THE
AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

CONTENTS

VOLUME TWENTY-ONE NUMBER 6

NOVEMBER-DECEMBER

1955

Page

A Flame Photometric Method for Calcium Determination by Direct Serum Dilution and Comparison with the Permanganate Titration Method
by Bernice Theissen Elert, B.S., M.T. (ASCP).297

A Study of Methods Used in the Identification of Candida Albicans
by Leanor D. Haley, Ph.D., M.T. (ASCP) and Mary Helen Stonerod, M.T. (ASCP).....304

Protein Standard from Pooled Sera
by Cleola M. Wurth, M.T. (ASCP).....309

The Role of Calcium Salt in the Kolmer Complement Fixation Test for Syphilis
by Eleanor Stackhous M.T. (ASCP).....311

The Role of Lymphocytes and Plasma Cells in the Formation of Antibodies
by Vernetta Hidde, M.T. (ASCP).....315

Medical Technologists in Canada
by D. W. Penner, M.D......321

The Use of a Marginal Punch Card in the Medical Laboratory for Routine Combined Reporting, Filing, Financial, Accounting and Statistical Purposes
by Elmer C. Sanborn, B.A., M.T. (ASCP).....326

Biological Hazards of Common Laboratory Procedures—I. The Pipette
by Morton Reitman, Ph.D., and G. Briggs Phillips, B.S......338

Biological Hazards of Common Laboratory Procedures—II. The Hypodermic Syringe and Needle
by Everett Hanel, Jr., B.S., and Robert L. Alg, B.S......343

Among the New Books.....303, 308, 314, 320, 325

Abstracts 347

The Gavel 350

From Our Readers.....337, 350

The Board of Schools.....351

Announcements .....351, M (Adv. Sec.)

The American Journal of Medical Technology is owned by The American Society of Medical Technologists. It is published bi-monthly. The volume begins with the January issue.

American Society of Medical Technologists

BOARD OF DIRECTORS

President: Barbara Isbell, 3725 Oleander Drive, San Diego 6, California
President-elect: C. Patton Steele, Box 1020, Bismarck, North Dakota
Treasurer: Mrs. Kathryn Dean, 1312 Silverthorne Road, Baltimore 12, Maryland
Secretary: Ellen Anderson, Pathology Dept., Med. School, Univ. of North Carolina, Chapel Hill, North Carolina
Ruth Heinemann, 3436 - 47th Ave. South, Minneapolis, Minnesota
Sister Mary Simeonette (Savage), Clinical Laboratory, Memorial Hospital, Chattanooga, Tennessee
Mrs. Elsa Kumke, 7726 East Jefferson, Detroit 14, Michigan
Anna Bell Ham, 1190 South Alhambra Circle, Coral Gables, Florida
Leanor Haley, Ph.D., 310 Cedar Street, New Haven, Connecticut
Rose Hackman, 842 Garfield Street, Denver, Colorado
Ruth Hovde, 515 Delaware Ave., S. E., Apt. 202, Minneapolis, Minnesota

ADVISORY COUNCIL OFFICERS

Chmn.: Jeanne Magnani, 6400 Elliott Place, Hampshire Knolls, Hyattsville, Md.
Vice Chairman: Emily E. McAdory, 220 Deadrick Street, Bessemer, Alabama
Recording Secretary: Ruth Feucht, 429 No. Lombard, Oak Park, Illinois
Asst. Recording Secretary: Sister Ann Marie Skinner, St. John's Hosp., 307 S. Euclid. St. Louis 10, Missouri
Corresponding Secretary: Joyce Wagner, 7017 Cedar Street, Wauwatosa 13, Wisc.

EXECUTIVE SECRETARY

Rose Matthaei, Suite 25, Hermann Professional Building, Houston 25, Texas

COMMITTEE CHAIRMEN

Finance: Anna Wassell, 1644 Northbourne Road, Baltimore 12, Maryland
Public Relations: Elizabeth O'Connor, 2203 Ridge Avenue, Evanston, Illinois
Education: Mrs. Jacqueline Bahrenburg, St. Luke's Hospital, Spokane, Washington
Standard and Studies Committee: Thelma Wilson, #4 Fairview Apts., South St., Bluefield, West Virginia
Membership Committee: Nila Maze, 6106 Carvel, Apt. C-3, Indianapolis 20, Indiana
Legislation: Lucille Larson, Tacoma General Hospital, Tacoma, Washington
Constitution & By-Laws: Marian Anderson, 1765 Laurel Avenue, St. Paul 4, Minn.
Recruitment: Audrey Murphy, 5444 Queens Avenue, St. Louis 15, Missouri
Research: Henrietta Lyle, Washington County Hospital, Hagerstown, Maryland
Nominations: Genevieve Stout, V. D. Research Lab., Box 285, Chamblee, Georgia
Civil Service-Armed Services: Ruth Heinemann, 3436-47th St. South Minneapolis 6, Minnesota
Insurance: Mrs. Elsa Kumke, 7726 East Jefferson, Detroit 14, Michigan
Board of Schools: Sr. M. Clare Heath, St. Clare's Hospital, 415 W. 51st St., New York 19, N. Y.

AFFILIATED SOCIETY PRESIDENTS

William M. DeLoach, Cloverdale Gr., Tuscaloosa, Alabama
Alice Timpe, c/o Alaska Native Service Hosp., Anchorage, Alaska
Mr. Harry N. Long, 2827 West Montebello, Phoenix, Arizona
Lila L. Church, 2116 Orange Street, No. Little Rock, Arkansas
Clara Brodsky, 340 Norwich, Los Angeles 48, California.
Lois Casperson, 2 East Boulder, Colorado Springs, Colorado
Carl Ritter, 20 Pearl Hill St., Milford, Connecticut
Vinna S. Ferrell, 1212 Delaware Avenue, Wilmington, Delaware
Jeanne Magnani, 6400 Elliott Place, Hyattsville, Maryland, (District of Columbia)
Philip G. Cash, 2535 1st Avenue N., St. Petersburg, Florida
Willard Jurney, Gready Memorial Hospital, Atlanta, Georgia
Lydia Martens, c/o Children's Hospital, Honolulu, Hawaii
Claire Lyons, Box 728, Caldwell, Idaho
Ruth Feucht, 427 No. Lombard, Oak Park, Illinois
Martha Winstead, 111 W. Bartlett, South Bend, Indiana
Clarence Gross, 1642 37th, Des Moines, Iowa
Virginia Hocker, 132 North Knox, Topeka, Kansas
Ruth Robinette, 3004 Bath Avenue, Ashland, Kentucky
Anna Persich, 2011 Perdido St., New Orleans, La.
Marilyn Sherman, North Scarboro, Maine
Sr. Paula Marie Phelan, R. S. M. Mercy Hospital, Baltimore, Maryland
Mrs. Helen Wiot, 39 Davis Street, Wollaston 70, Massachusetts
Mrs. Mary McPhee Scott, 13587 Monte Vista, Detroit 38, Michigan
Verna Rausch, 5609 Fremont Street, Minneapolis, Minnesota
Alida M. Bos, 333 Illinois Avenue, Vicksburg, Mississippi
Helen Alig Dawes, 7222 Charlotte, Kansas City, Missouri
Catherine Reid, 1514 2nd Avenue North, Great Falls, Montana
Dorothy E. Jahn, 800 South 13th St., Lincoln, Nebraska
Mrs. Julie Waller, 1155 Jones St., Reno, Nevada
Robert P. Grovenstein, RFD #1, Rochester, New Hampshire
Mrs. Catherine Milos, 631 Ridgewood Road, Oradell, New Jersey
Sylvia Saarnijoki, 310 Marshall St., Syracuse 10, New York
Mary Elizabeth Motsinger, 2428 South Main Street, Winston-Salem, North Carolina
Mrs. Margaret H. Saumur, Path. Dept., Univ. of N. D., Grand Forks, North Dakota
Genevieve Goodwin, 125 Prospect St., Dover, Ohio
Billie Scarbrough, 601 E. Broadway, Cushing, Oklahoma
Mary Elizabeth Baptist, 211 North West 21st Ave., Portland, Oregon
Romayne A. Teufel, 1167 Pemberton St., Pittsburgh 12, Pennsylvania
Mary Eileen Leonard, 907 Rutledge Avenue, Charleston, South Carolina
Sister Mary Vincentia, St. John's Hospital, Huron, South Dakota
Deane Brown, 1151 Eleanor St., Knoxville, Tennessee
Ruth Morris, Box 1719, Ft. Worth, Texas
Colleen Hamilton, 1125 Alpine Place, Salt Lake City 5, Utah
Alice Laughlin, Nutrition Research Dept. Medical College, Univ. of Vermont, Burlington, Vermont
Rosemary Henretty, Johnston Memorial, Abingdon, Virginia
Warren P. Brown, Good Samaritan Hospital, Puyallup, Washington
Mrs. Marjorie Plymale, 1439 Sixth Avenue, Huntington, West Virginia
Joyce Wagner, 7017 Cedar Street, Wauwatosa, Wisconsin
Dorothy Thomas, 418 W. 19th St., Cheyenne, Wyoming

The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 21 NOVEMBER-DECEMBER NUMBER 6

A FLAME PHOTOMETRIC METHOD FOR CALCIUM DETERMINATION BY DIRECT SERUM DILUTION AND COMPARISON WITH THE PERMANGANATE TITRATION METHOD*†

BERNICE THEISSEN ELERT, B.S., M.T. (ASCP)
Biochemist, Instructor in Chemistry
Charles T. Miller Hospital, St. Paul, Minnesota

Blood and urine calciums have probably been determined most frequently by precipitation as calcium oxalate followed by the titration of the oxalate by the Clark-Collip[8] modification of the Kramer Tisdall method.[7] This method at best can be done in three hours using a short precipitation time,[10] but this is too long when a doctor must decide whether a low calcium is a factor in the cause of convulsions. This is often encountered in newborns and prematures, and treatment of such patients begins without benefit of calcium levels. This paper presents a comparison of the oxalate precipitation oxidimetric titration method for blood calcium[7,8,9,10] and the direct serum dilution method with use of the Beckman DU spectro-flame photometer with the photomultiplier attachment. Since this requires no preliminary preparation of serum samples, the direct serum dilution method can be done very rapidly. As an example, eight serum samples in addition to the standard curve were run in this laboratory in less than twenty minutes.

In addition to the time consumed, the permanganate titration method has other limitations. The accuracy realized is dependent upon the preliminary standardization of sodium oxalate, and several ml. of serum are necessary to do the analysis. The non specificity of the oxidizing agent and the precipitation of oxalates other than calcium oxalate, such as magnesium oxalate, may give erroneous results. These discrepancies which occur when using the calcium oxalate precipitation method may be

* From the Department of Pathology, Charles T. Miller Hospital, St. Paul, Minn.
† Third ASMT Award, read before ASMT, New Orleans, La., June, 1955.

eliminated by a direct measurement of calcium with a flame photometer. Many such procedures have been suggested. Kapuscinski and his co-workers[13] and Mosher et al.[4] measured the luminosity of calcium after wet ashing the serum and separating the calcium as the phosphate. This procedure at best takes five hours. Sevringhaus and has associates[14] have employed trichloracetic acid protein precipitation prior to atomization of the sample, but this is also time consuming. Others have used direct dilution of serum. The direct serum dilution method described by Winer and Kuhns[18] employing an organic solvent consisting of 70 per cent acetone, 20 per cent glacial acetic acid, and 10 per cent of a 2 per cent Sterox solution by volume, proved unsuccessful in our hands, and we were unable to reproduce their results. The organic solvent was not easy to work with and repeatedly blew out the flame unless an aqueous diluent was added to the beaker before lowering it from the atomizer burner. Full credit is given to M. F. Hritz[1] for his work in employing the use of 10 per cent isopropyl alcohol as a propellant in 1-25 ml. direct serum dilutions with no preliminary treatment of the sample. The alcohol serves to accentuate the acetylene flame. His method in our hands yielded results 0.3 to 0.7 mEq/1 too low when compared to the titration method, but the use of the alcohol was extremely successful and we have utilized it in the work reported in this paper.

In any of these methods, however, inconsistencies may be shown due to age and condition of the serum. Chen and Toribara[3] have shown that in aged serum which is partially denatured or cloudy, the protein which precipitates out carries with it part of the calcium. In our study a series of serums were run while fresh, then allowed to stand in the refrigerator for a few days, and when run again, we found the results were lower, as expected. All results presented in this report were done on freshly drawn serum.

Preparation of the Standard

It is well known that sodium and potassium will increase the intensity of the spectral lines of calcium. The calcium carbonate standards employed contained sodium and potassium in amounts similar to the diluted serum sample in order to correct for enhancement of the calcium spectral bands. During preliminary work, magnesium and phosphorous were also added to the standards to correct for any depression of the calcium bands,[3,4] but since no effect was noted at 422.7 mu. (peaked), this was no longer continued. Chen and Toribara[3] have demonstrated that phosphate depression of calcium emission occurs in absence of protein. If then, the analysis is carried out on diluted serum, the phosphate depression is prevented.

A stock standard of desiccated calcium carbonate (Iceland

Spar) was made so that 1 ml = 2 mgs. calcium (2.4966 grams $CaCO_3$ were dissolved in 5 ml. of concentrated HCl and diluted to 500 ml. with pyogen free water). Four ml. of the stock standard were diluted to 100 ml. with pyogen free water to coincide with the 1-25 ml. serum dilutions. This was the intermediate calcium standard. The working standards were made by pipetting 3, 4, 5, 6, and 7 ml. of intermediate standard into 100 ml. volumetric flasks which corresponded to 3 through 7 mEq calcium per liter respectively. To each 100 ml. volumetric flask was added 4 ml. of potassium as KCl (6.1 mEq/1) and 4 ml. of sodium as NaCl (143 mEq/1). Ten ml. of isopropyl alcohol were added to all flasks and then diluted to volume with pyogen free water.

Preparation of the Sample

One ml. of fresh serum was added to an acid-washed[1] 25 ml. volumetric flask. Ten ml. of pyogen free water were added, followed by 0.1 ml. of 20 per cent HCl (to arrive at a final pH of 3.5-4.5)[2] and 2.5 ml. of isopropyl alcohol. These were diluted to volume with pyrogen free water. A dilution using less serum, 0.2 ml. diluted appropriately to volume in a 5 ml. volumetric flask, was very satisfactory. The protein content in these dilutions was not sufficient to cause any plugging of the atomizer burner.

Sample Analysis

The operation of the Beckman DU has been described in detail,[11,12] and the usual precautions regarding selection of optimal pressures of oxygen and gas for the individual instrument in use were adhered to. In the vicinity of the peak, relatively large variations in oxygen pressure cause relatively small changes in transmittance readings. It is in this less sensitive zone of pressures that transmittance readings are most accurate.

The settings optimal for calcium determination in this laboratory were:

Oxygen	9 lbs psi
Acetylene	4 lbs psi
Wave Length	422.7 mu (peaked)
Slit Width	0.07 mm
Selector Switch	0.1
Resistor	22 megohms

The photomultiplier tube was positioned and the photomultiplier switches were set at sensitivity 4 and zero suppression 1. When the photomultiplier tube is employed, the sensitivity may be regulated, not only with the sensitivity knob on the monochromator, but also by changing the power passing into the

[1] Cleaning solution, 100 grams of sodium dichromate dissolved in water to 9 lb. technical grade concentrated sulfuric acid.
[2] Twenty ml. of concentrated reagent grade HCl diluted to 100 ml. with distilled water.

photomultiplier tube. The slit width was selected in accordance with the available emission intensity and the required resolving power. In general, the narrowest slit giving adequate readings should be used. These settings, however, are arbitrary and must be determined for each instrument. The three wavelengths used for detection of calcium, 422.7 mu., 622 mu., and 554 mu., were all examined with the conclusion that the best resolving power and most sensitivity with least spectral interference on our instrument was achieved at 422.7 mu. (peaked).

The standards and samples were transferred to 5 ml. Beckman beakers. The instrument was peaked for Ca while burning the 7 mEq/l standard. A standard curve, 3 mEq/l through 7 mEq/l, was run each time, followed by the serum samples which were then bracketed by the standards. In all cases duplicate readings were taken of all samples. After every two serum samples were atomized, water was aspirated to prevent the protein from plugging the burner. A tendency was noted for the flame to blow out when the beaker was lowered from the atomizer burner, but if relit and the oxygen and acetylene pressures resumed, the standard curve did not shift. With slow manipulation of the sample positioning arm, this tendency was no longer evident.

Examples of transmittance readings on standards were:

7 mEq/l calcium	75.0% T	Transmittance
6 mEq/l calcium	69.6% T	
5 mEq/l calcium	64.6% T	
4 mEq/l calcium	59.2% T	
3 mEq/l calcium	53.8% T	
0 mEq/l calcium	35.0% T	(flame background)

This curve was completely reproducible and the scale was linear with respect to calcium providing other variables were held constant. The flame background was measured during aspiration of pyrogen free distilled water containing 10 ml. of isopropyl alcohol per 100 ml. This transmittance reading could have been subtracted from that obtained during aspiration of the samples to get the intensity value for calcium. However, since the serum samples were bracketed by standards, the flame background was disregarded because linear relationship was proven in the standards, and a curve was plotted, with transmittance as ordinate and concentration as abscissa, from which the serum sample concentrations were read. The approximate spread obtained for Ca was in the order of 1 transmittance division per 0.185 mEq/l of calcium.

Advantages of the flame photometry method were the use of small amounts of serum for analyses and the rapidity with which results were obtained. These are gained without sacrifice of accuracy as can be seen in Table I which presents a comparison of the two methods.

Table I
Comparison of Serum Calcium Values (mEq/L) and Proteins Observed

Ca by Permanganate Titration Method (a)	Ca by Flame Photometry (b)	Difference (b-a)	Total	Gms. /100 ml. Alb.	Protein Glob.
3.85	3.75	—.10	4.9	2.1	2.8
4.10	4.10	....	....	....	....
4.53	4.45	—.08	7.5	2.0	5.5
4.56	4.50	—.06	9.6	3.1	6.5*
4.65	4.83	+.18	4.5	1.6	2.9
4.90	4.85	—.05	5.6	3.2	2.4
4.90	5.08	+.18	5.9	2.7	3.2
5.05	5.06	—.01	6.2	3.4	2.8
5.05	5.15	+.10	5.8	2.8	3.0
5.10	5.10	....	....	....	....
5.15	5.16	+.01	6.2	3.9	2.3
5.15	5.25	+.10	6.6	3.4	3.2
5.20	5.16	—.04	6.8	3.8	3.0
5.20	5.20	....	....	....	....
5.24	5.23	—.01	6.9	3.3	3.6
5.27	5.18	—.09	6.9	3.4	3.5
5.40	5.30	—.10	6.7	3.7	3.0
5.55	5.60	+.05	7.2	3.9	3.3
5.60	5.55	—.05	6.8	3.8	3.0
5.65	5.58	—.07	7.0	3.6	3.4
5.80	5.89	+.09	....	....	....
5.90	5.82	—.08	....	....	....
6.10	6.10	....	....	....	....
6.40	6.40	....	....	....	....
8.45	8.29	—.16	6.0	4.0	2.0
Mean[1] 5.35	5.32	Av. ±.083	....	....	....

[1] 46 cases were studied, but due to lack of space, only 25 are presented. The mean and average difference were based on all 46 cases.
* Multiple Myeloma.

It is seen that excellent agreement was obtained between the permanganate titration method and flame photometer serum dilution method. The average deviation was ± 0.083.

The idea is now accepted that Ca is bound to plasma proteins at physiological pH, and Carr[2] using bovine albumin has shown that there is no protein bound calcium when the pH is lowered to 4.5 but that binding increased as the pH is raised. Evidence

Table II
Binding of the Calcium Ion by Plasma Protein at Various pH Values
(Serum Ca Values in mEq/1 by flame photometry method)

pH 1.5-2.5	pH 3.5-4.5	pH 6.5-7.5	pH 8.5-9.5
	4.50	3.90*	
	5.00	4.45	
	5.00	4.83	
5.06	5.06	4.62	4.45
5.15	5.15	4.73	
	5.16	4.60	
	5.23	4.75	
5.22	5.23	4.85	4.62
5.18	5.25	4.87	4.65
5.58	5.58	5.08	4.98
	5.59	5.22	
	5.87	5.30	
	6.00	5.12	
	8.29	7.67	

* Multiple Myeloma.

that this is also true of human plasma protein is presented in Table II.

These results show that there is a large increase in the binding of calcium ions as the pH is increased above the isoelectric point.

In order to exemplify reproducibility and recovery of calcium in the analyses of human serum, each of six serum samples was analyzed in duplicate with and without addition of 1 mEq/1 Ca as seen in Table III.

Table III

Recovery of Calcium (mEq/1) in serum Dilutions by Flame Photometry After Addition of 1 mEq/1 Ca

Serum Ca Present (mEq/1)	Ca Found After Addition of Recovery (mEq/1)	Ca Recovered (mEq/1)
5.23	6.20	0.97
5.06	6.04	0.98
5.25	6.25	1.00
5.58	6.56	0.98
5.18	6.18	1.00
4.45	5.45	1.00

The calcium recoveries were excellent as shown by an average recovery of 0.988 mEq/1 Ca.

Summary

1. A flame photometric method for quantitative determination of serum calcium is described using a 1-25 ml. dilution of serum in 10 per cent isopropyl alcohol at a pH of 3.5-4.5.
2. Comparative data is presented between the direct serum dilution method and the permanganate titration method showing excellent agreement. The rapidity gained and the accuracy achieved make the flame photometric method the method of choice.

ACKNOWLEDGMENT

The author is indebted to Miss Mavis Hawkinson, assistant chemist, Charles T. Miller Hospital, for her valuable technical assistance.

BIBLIOGRAPHY

1. Hirtz, Michael F., M. S., chemist: Personal Communication. Oak Park Hospital, Oak Park, Illinois.
2. Carr, C. W., Studies on the Binding of Small Ions in Protein Solutions with the Use of Membrane Electrodes. The Binding of Calcium Ions in Solutions of Bovine Serum Albumin. Arch. Biochem. & Biophys. 43: 147 (1953).
3. Chen, P. S. Jr., and Toribara, T. Y.: Determination of Calcium in Biological Materials by Flame Photometry. Anal. Chem. 25: 1642 (1953).
4. Mosher, R. E., Itano, M., Boyle, A. J., Myers, G. B., and Iseri, L. T.: The Quantitative Estimation of Calcium in Human Plasma by Flame Photometry. Am. J. Cl. Path. 21: 75 (1951).
5. Smith, R. G., Craig, P., Bird, E. J., Boyle, A. J., Iseri, L. T., Jacobson, S. D., and Myers, G. B.: Spectrochemical Values for Sodium, Potassium, Iron, Magnesium, and Calcium in Normal Human Plasma. Am. J. Cl. Path. 20: 263 (1950).

6. Chen, P. S. Jr., and Toribara, T. U.: Some Errors in the Determination of Calcium in Aged Blood Serum. Anal. Chem. 26: 1967 (1954).
7. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum. J.B.C. 47: 475 (1921).
8. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium with a Suggested Modification. J. B. C. 63: 461 (1925).
9. Sendroy, J. Jr.: Determination of Serum Calcium by Precipitation with Oxalate. J. B. C. 152: 539 (1944).
10. Elert, B. T.: A Comparative Study of Precipitation Time and Temperature Factors Affecting Blood and Urine Calciums. Am. J. Med. Tech. 20: 263 (1954).
11. Gilbert, P. T., Hawes, R. C., and Beckman, A. O.: The Beckman Flame Photometer. Anal. Chem. 22: 772 (1940).
12. Mosher, R. E., Bird, E. J., and Boyle, A. J.: Flame Photometric Determination of Calcium in Bruicite and Magneside. Anal. Chem. 22: 715 (1950).
13. Kapuscinski, V., Moss, N., Zak, B., and Boyle, A. J.: Quantitative Determination of Calcium and Magnesium in Human Serum by Flame Spectrophotometry. Am. J. Cl. Path. 22: 687 (1952).
14. Sevringhaus, J. W., and Ferreble, J. W.: Calcium Determination by Flame Photometry. J. B. C. 187: 621 (1950).
15. Zak, B., Mosher, R. E., Boyle, A. J.: A Review on Flame Analysis in the Clinical Laboratory. Am. J. Cl. Path. 23: 60 (1953).
16. Murayama, M.: A Rapid Method for Serum Calcium Determination. J. Lab. and Cl. Med. 33: 906 (1948).
17. Denson, J. R.: Flame Photometric Determination of Electrolytes in Tissue and of Calcium in Serum. J. B. C. 209: 233 (1954).
18. Winer, A. D., and Kuhns, D. M.: Calcium Determination by Flame Spectrophotometry. Am. J. Cl. Path. Tech. Bull. 23: 255 (1953).

AMONG THE NEW BOOKS

CLINICAL PATHOLOGY IN GENERAL PRACTICE: from the British Medical Journal, 1953-1954. British Medical Association, B.M.A., House, Tavistock Square, London, W. C. I., England. 1955. 39 Articles. 321 pages. Charts. Illustrations 21. (approx. $3.00.)

This volume is designed primarily for the physician. It is a collection of articles that appeared originally in the British Medical Journal in a Refresher Course series for General Practitioners. The articles have been brought up to date. While specific material is English in origin, most can be applied to American practice as well.

PHYSICIANS' OFFICE ATTENDANTS' MANUAL: by Henry B. Gotten, M.D., Associate Professor of Medicine, University of Tennessee (office work section), and Douglas H. Sprunt, M.D., Professor of Pathology, University of Tennessee (laboratory section), Charles C. Thomas Company, Springfield, Illinois. 1955. 93 pages, 35 illustrations. $3.75.

The book can be used by those who feel the scarcity of medical technologists warrants the employment of less technically trained personnel in the physician's office.

Many of the points discussed could well be taken by any employee, whether in the laboratory or in the hospital. Such details as the qualifications of a Medical Assistant, record keeping, telephone calls, and referring of patients, etc., would be especially valuable. Many of the so-called "simple" laboratory techniques are given.

A STUDY OF METHODS USED IN THE IDENTIFICATION OF CANDIDA ALBICANS*

LEANOR D. HALEY, Ph.D., M.T. (ASCP), and
MARY HELEN STONEROD, M.T. (ASCP)

Department of Microbiology—Yale University School of Medicine
and the
Nancy Sayles Day Mycology Laboratory

The development of chlamydospores by *Candida albicans* on corn meal agar, or a variant thereof, is a characteristic which enables rapid identification of this species. Failure to develop these chlamydospores by this fungus necessitates a method for identification requiring approximately ten days.

The routine use of corn meal agar is a result of Benham's[1] observation that Candida species, when cultured on this medium, could be differentiated from other yeast-like fungi by the development of pseudohyphae, and that *Candida albicans* frequently developed chlamydospores. Unfortunately, the development of these chlamydospores is somewhat irregular with the result that many variations of corn meal agar have been introduced, always with the hope that the production of these spores by *Candida albicans* will be consistent.

Nickerson and Mankowski[2] have shown that the presence of glucose or cysteine in corn meal agar will suppress the production of both pseudohyphae and chlamydospores. As a result of their studies, they developed a purified polysaccharide medium which contained ammonium sulfate as the nitrogen source. A clinical trial of this medium made by a diagnostic laboratory indicated that it would induce the production of pseudohyphae and chlamydospores of a large number of strains of *Candida albicans*. This laboratory reported that of 247 results obtained, 212 were identical on corn meal agar and the polysaccharide medium: "19 positive for *Candida albicans*, 53 positive for Candida species, 33 overgrown by non-pathogenic fungi, while 107 were negative." It is interesting to note that among the 35 remaining instances, variation in the production of chlamydospores and pseudohyphae was noted: 14 fungi being identified as *Candida albicans* on the polysaccharide medium and only 9 being identified as *Candida albicans* on the corn meal agar, with the remainder being classified as either other species of Candida or fungi falling into other genera.

This instance of variation among strains of *Candida albicans* reported by the above laboratory is seen by many other laboratories. It is because of this variation that mycologists are willing to try any medium that might indicate improvement over the old corn meal agar, or any technique in inoculation of these media that might enhance the development of chlamydospores.

* Received for publication July, 1955.

Weld[3] has described a method for identifying *Candida albicans* using Levine's eosin-methylene blue agar. This medium is inoculated by streaking with several loopfuls of a saline suspension of the Candida species. Following incubation for 18-24 hours at 37° C. in an atmosphere of approximately 10% CO_2, *Candida albicans* will be characterized by the presence of colonies with spidery or feathery mycelium. In her report, Weld states that with the exception of *Candida stellatoidea*, other species of Candida do not produce mycelium under these conditions.

It is the purpose of this paper to present a careful study made with 18 strains of *Candida albicans* freshly isolated from human material and identified by the method of Conant and his associates.[4] Four different media and four different methods of inoculating these media have been investigated.

Methods

Four media were used in this study. They were, (1) a polysaccharide medium,[2*] (2) Zein agar,[5**] (3) corn meal agar without dextrose,[***] and (4) Levine's eosin-methylene blue agar. One hundredth of a percent trypan blue was added to the corn meal and Zein media.

All of the media were prepared on the same day and then inoculated by one individual. The inocula used were taken from beef extract agar slants.

The polysaccharide medium, Zein and corn meal agar were inoculated in the following manner:

a. agar in petri dishes were cut twice with a bacteriological probe containing the inoculum; one of the two cuts was covered with a sterile 22 x 40 mm. coverslip.
b. a saline suspension (No. 2 MacFarlane nephelometer) of the inoculum was streaked over the surface of the media; two plates per medium were used with a sterile 22 x 40 mm. coverslip being placed over the last streaks made on the second plate.

These cultures were incubated at room temperature and examined for pseudohyphae and chlamydospores at 24, 48 and 72 hour intervals.

Eosin-methylene blue medium was inoculated according to Weld's method and incubated at 37° C. in 10% CO_2 and aerovically.

Polysaccharide and Zein media were inoculated by the cut method and then incubated at room temperature in the presence of 10% CO_2.

* Chlamydospore agar—Difco Laboratories.
** Zein—Nutritional Biochemical Corporation, Cleveland 28, Ohio.
*** Yellow corn meal used.

Results

Table 1 summarizes the results obtained with corn meal, Zein, and polysaccharide media when each was inoculated by 4 different methods.

Table I
Production of Chlamydospores by *Candida Albicans* on Corn Meal, Polysaccharide and Zein Media when innoculated by four different methods

MEDIUM	STREAKING OF SURFACE WITH LOOP		AGAR CUT WITH A BACTERIOLOGICAL LOOP	
	Inoculum Covered With Coverslip	Inoculum Not Coverslipped	Cuts Coverslipped	Cuts Not Coverslipped
Corn meal agar.....	6/18*	6/18	8/18	13/18
Polysaccharide agar..	9/18	9/18	7/18	7/18
Zein agar.....	13/18	6/18	9/18	8/18

* Number of Strains Producing Chlamydospores / Total number of strains studied.

When the figures contained in this table were subjected to statistical analysis it was found that there was no significant difference between the media used or in the methods of inoculation. The important observation is that no single medium or method used was able to induce the production of chlamydospores by all the strains of *Candida albicans*.

Table II indicates very clearly that the more methods used in inoculating a medium the greater is the chance of inducing production of chlamydospores. An additional method has been included within this table—the use of the Reddell[6] slide culture technique. This slide culture technique had no advantage over any of the other methods used for producing chlamydospore formation.

Table II
Production of Chlamydospores by *Candida Albicans* Using One or More Methods

MEDIUM	By One Method Only	By Two Methods Only	By Three Methods Only	By Four Methods Only	By Five Methods Only	Total Number Strains Producing Chlamydospores
Corn meal agar.....	1/18*	2/18	3/18	6/18	6/18	18/18
Polysaccharide agar	2/18	5/18	1/18	2/18	4/18	14/18
Zein agar.....	4/18	3/18	1/18	2/18	5/18	15/18

* Number of Strains Producing Chlamydospores. / Total number of strains studied

Although corn meal agar, when inoculated by five methods, caused production of chlamydospores by all 18 strains of *Candida albicans* used in this study, there is actually little difference between the media used and the development of chlamydospores.

Of 18 strains of *Candida albicans* inoculated to eosin-methylene blue agar, 14 strains demonstrated marked feathering of the colonies, 3 strains showed only slight feathering which could be missed if the plates were read hastily, and 1 strain failed to produce any colonies with a feathery border. No strains of *Candida albicans* developed a feathery appearance when this medium was incubated aerobically.

There was no significant difference in the production of chlamydospores on Zein and polysaccharide media incubated aerobically or in 10% CO_2.

Discussion

Strains of *Candida albicans* vary greatly in their ability to produce chlamydospores and it is this factor which makes it difficult to find one medium or even one method which will induce the development of these spores. This study would serve to emphasize this point, since no significant difference in the methods of inoculation or of the media inoculated could be detected. It would appear that the more methods used in inoculating a medium, the better is the chance of acquiring production of chlamydospores.

Throughout this entire study, we were impressed by the fact that the amount of inoculum used is of the utmost importance. If the inoculum was too thick, one was likely to see no or little pseudohyphae or chlamydospores. The thinner the inoculum the better the chances of obtaining growth characteristic for *Candida albicans*.

Although these cultures were read over a three-day period, the majority of the pseudohyphae and chlamydospores were noted within twenty-four hours; occasionally these spores were noted for the first time at the end of 48 hours but on only one occasion were chlamydospores noted at 72 hours having been absent previously.

If the technique of using eosin-methylene blue medium for differentiating *Candida albicans* from other species of Candida should continue to be as satisfactory as is indicated by this study, it will indeed be an asset to the laboratory, for it enables the technologist to identify this fungus in 18 hours.

As a result of this study, the following routine has been set-up in the Mycology Laboratory of the Yale-New Haven Medical Center: the yeast-like organism is subcultured from the original medium to eosin-methylene blue agar and to beef extract agar. Growth on the eosin-methylene blue medium is noted for feathering; a small amount of the growth on the third generation from beef extract agar is subcultured to Zein agar; if chlamydospores are not developed the fungus is then studied for its fermentation reactions.

REFERENCES

1. Benham, R. W.: Certain Monilias Parasitic on Man. Their identification by morphology and by agglutination. Journal of Infectious Diseases, 49:183-215, 1931.
2. Nickerson, W. J. and Mankowski, Z.: A polysaccharide medium of known composition favoring chlamydospore formation in *Candida albicans*. Journal of Infectious Diseases, Vol. 92:20-25, 1953.

3. Weld, J. T.: *"Candida albicans*: Rapid identification in pure cultures with carbon dioxide on modified eosin-methylene blue medium." A.M.A. Archives of Dermatology et Syphilis, 66:691-694, 1952.
4. Conant, N. F., Smith, D. T., Baker, R. D., Calloway, J. L., and Martin, D. S.; Manual of Clinical Mycology, Second edition, W. B. Saunders Company, Philadelphia, 1954.
5. Reid, J. D.: A simple clear medium for demonstration of chlamydospores of *Candida albicans*. Technologists Bulletin, 23:188, 1953.
6. Reddell, R. W.: Permanent stained mycological preparations obtained by slide culture. Mycologia 42:265-270, 1950.

APPENDIX

Since the completion of studies reported in this paper, 45 additional isolates of Candida species have been made in this laboratory. Twenty-five were *Candida albicans* and two were *Candida stellatoidea*. Of these 27 strains, 13 of *Candida albicans* and both of the strains of *Candida stellatoidea* developed characteristic growth on eosin-methylene blue medium and produced chlamydospores on Zein medium. Three strains of *Candida albicans* failed to produce chlamydospores but did develop characteristic growth on eosin-methylene blue medium; 6 other strains did not show the feathery colonies on eosin-methylene blue medium but did develop chlamydospores on Zein agar. Three strains of *Candida albicans* were identified by fermentation reactions. Two strains of *Candida tropicalis* developed the characteristic feathery colony of *Candida albicans* when cultured on eosin-methylene blue medium.

AMONG THE NEW BOOKS

BIOCHEMISTRY: AN INTRODUCTORY TEXTBOOK: by Felix Haurowitz, Dr. Med. (Prague), Dr. rer. nat. (Prague), Professor of Chemistry, Indiana University, Bloomington, Ind. John Wiley and Sons, Inc., 1955. 485 pages. Tables and illustrations. $6.75.

Although this is a textbook and is primarily devoted to general biochemistry, there is still much of value to the person in the medical field. Throughout the volume there is material on respiration, digestion, and Blood Chemistry. The final chapter is on biochemistry of the human body fluids. This will be a boon to the Medical Technologist whose training in some of the fundamentals has been neglected.

INTRODUCTION TO PARASITOLOGY: 9th Edition. Asa C. Chandler, M.S., Ph.D., Professor of Bacteriology, Rice Institute, Houston, Texas. Former Officer-in-Charge, Hookworm Research, Laboratory, School of Tropical Medicine and Hygiene, Calcutta, India. John Wiley and Sons, Inc., New York. 1955. 799 pages, 257 illustrations. $8.50.

This edition emphasizes the value of revision according to subjects currently in the forefront of scientific research. There is new data on the conquest of syphilis by penicillin, the end of malaria as an endemic disease in the United States and parts of Europe, Babesiidae, Toxoplasma, the physiology of helminths and immunology in helminthic infections, techniques for tapeworm infections, and invasion routes of animal ascarids.

The sections on onchocerciasis, the discussion of larvae of foreign species of filariae, and an account of the species, habits, and vector potential of various species of Simuliids to onchocerciasis and of tsetse flies to African Trypanosomiasis are either enlarged or rewritten. Approximately half of the 257 illustrations have been improved or recast.

The entire subject matter has been reappraised and brought up to date.

PROTEIN STANDARD FROM POOLED SERA

CLEOLA M. WURTH, M.T. (ASCP)
St. Mary's Hospital, Decatur, Illinois

One of the problems confronted in the accurate determination of total serum protein values is securing a reliable yet inexpensive standard. Many of the commercial companies now produce standards that are comparatively stable and reasonably inexpensive, but we feel that this is an unnecessary expense to a laboratory which discards daily a large volume of human blood serum. In recent studies we have found that pooled sera will provide a very constant protein value.

By pooling the remaining clear, unhemolyzed, non-icteric sera from the serological tests and determining the protein value by the Biuret method of Weichelbaum;[1] we have found the protein reasonably constant at seven grams per 100 ml. Macro-Kjeldahl protein determinations performed on pooled sera gave a mean protein value of 6.99 grams percent which was used as a basis for calibrating the Beckman "B" spectrophotometer. Forty-seven pools of sera were then analyzed. The results gave a mean protein value of 7.0 grams percent with a standard deviation of ±0.15 grams % (Table I).

TABLE I

Total Protein Value of Pooled Sera

No.	Serum Samples in Pool	Protein Value Grams Percent	No.	Serum Samples in Pool	Protein Value Grams Percent
1.....	18	7.0	6.....	25	7.1
2.....	37	7.0	7.....	29	7.2
3.....	20	7.0	8.....	21	7.0
4.....	29	6.9	9.....	26	6.9
5.....	32	6.9	10.....	31	7.2

Mean: 7.02 gms%. (N = 47).
Standard Deviation: ±0.15 gms%.

Tests were also performed to determine the number of serum samples necessary for a "pool." Twenty was found to be the minimum number required to obtain a pool having a protein value of 7 grams percent (Table II). Smaller laboratories may not have this amount of serum readily available; this problem may be overcome by holding the serum in the refrigerator until more samples are available. We have been able to keep pooled sera under refrigeration for twenty days with no apparent change in the protein value. It is to be noted that no special effort was made to keep this sera sterile.

The use of the discarded serum from serology is to be stressed. It is felt that these samples afford a greater supply of nearly normal protein serum than the discard from the chemistry labo-

ratory. Also, the serum remaining from each of the serological tests is of a fairly equal volume and thereby avoids the error which could be produced by a large volume of either a high or a low protein sample.

TABLE II

A comparison of the protein values in pools of 10 samples against pools of 20 samples

POOLS OF TEN SAMPLES		POOLS OF TWENTY SAMPLES	
No.	gms% Protein	No.	gms% Protein
1.	7.4	1.	7.0
2.	6.7	2.	6.8
3.	7.0	3.	6.9
4.	6.3	4.	6.6
5.	8.2	5.	7.5
6.	7.3	6.	7.0
7.	7.0	7.	6.9
8.	7.8	8.	7.1
9.	6.9	9.	6.9
10.	7.2	10.	7.1
Mean:	7.28 gm%.	Mean:	6.98 gm%.
Standard Deviation:	±0.55 gm%.	Standard Deviation:	±0.24 gm%.

Pooled sera may also be used as a means of standardizing tests for spinal fluid proteins. A 1:100 dilution of pooled sera will produce a 70 milligram percent standard and a 1:200 dilution will produce a 35 milligram percent standard.

Summary: An inexpensive, reliable, and readily accessible method of procuring a protein standard has been presented. This standard is suitable for calibrating both serum and spinal fluid protein determinations.

REFERENCE

1. Weichselbaum, T. E.: An accurate and rapid method for the determination of protein in small amounts of blood serum and plasma. Am. J. Clin. Path., 16, 40 (1946).

ACKNOWLEDGMENT

The author wishes to express her appreciation to Dr. Welland A. Hause, Pathologist, St. Mary's Hospital, Decatur, Illinois, for his assistance in the preparation of this manuscript.

THE ROLE OF CALCIUM SALT IN THE KOLMER COMPLEMENT FIXATION TEST FOR SYPHILIS*

ELEANOR A. STACKHOUS, M.T. (ASCP)
740 Easton Road, Horsham, Penn.

During 1954, Dr. Alcor S. Browne, California Dept. of Health[1] suggested that the addition of 0.04 grams of $CaCl_2 \cdot 2H_2O$ per liter of Kolmer salt[2] would enhance the hemolytic activity of complement without materially effecting the reactivity in the quantitative tests for syphilis.

Dr. John A. Kolmer et al[3] further confirmed this finding, concluding that the preliminary titrations showed higher titers of both hemolysin and complement with calcium salt added than did the regular Kolmer salt. In the quantitative serum and spinal fluid tests the titers were somewhat higher with calcium salt than with regular Kolmer salt.

Both authors did find, however, a greater tendency to false positive and anticomplementary reactions with calcium salt than with regular Kolmer salt.

The following work was undertaken to evaluate these previous findings.

Technique

All preliminary titrations for hemolysin and complement activity were done in duplicate, using regular Kolmer salt and Kolmer salt plus 0.04 gms. $CaCl_2 \cdot 2H_2O$ per liter of distilled water. The sheep cells were washed in Kolmer salt but at the final packing they were divided into two lots. One lot of 2% cell suspension was made up in Kolmer salt, the other in Kolmer salt plus calcium salt. The antigen was Kolmer Cardiolipin (Sylvana Lab.). The 1:100 hemolysin was made up in Kolmer salt, but from this point on all dilutions of antigen, hemolysin and complement were made up in duplicate with regular Kolmer salt and Kolmer salt plus calcium salt.

Nearly all the sera were treated with sheep cells to absorb the native sheep amboceptor according to the Stackhous method.[4] All the tests were read at the end of 20-25 minutes after the second incubation in the 37° C water bath. On only one day was there a prolongation of the time it took for the tests with $CaCl_2$ to clear.

In all, 19 duplicate titrations were run with the following results.

---

* Second Scientific Products Foundation Award, Serology Division, 1955.

Table 1

Titrations	Hemolysin 2 Units in 0.5 cc.		Complement 2 Full Units in 1 cc.	
	Kolmer NaCl	Kolmer NaCl and $CaCl_2$	Kolmer NaCl	Kolmer NaCl and $CaCl_2$
3	1:4000	1:6000	1:37	1:60
1	1:4000	1:6000	1:37	1:50
1	1:4000	1:6000	1:33	1:60
1	1:4000	1:4000	1:30	1:50
1	1:4000	1:5000	1:37	1:50
2	1:4000	1:5000	1:30	1:100 plus
1	1:4000	1:5000	1:33	1:100 plus
1	1:4000	1:5000	1:27	1:60
1	1:5000	1:6000	1:33	1:50
1	1:5000	1:8000	1:33	1:50
1	1:5000	1:6000	1:43	1:60
1	1:3000	1:3000	1:37	1:50
1	1:3000	1:4000	1:30	1:50
1	1:3000	1:5000	1:33	1:60
1	1:2500	1:3000	1:37	1:50
1	1:2000	1:4000	1:30	1:37

In all but two instances the titers of hemolysin were from 1:500 to 1:3000 higher (1 to 3 tube difference) using the calcium salt. Likewise in all but one complement titration the titer was enhanced in the calcium salt solution, being 2-7 tubes higher. In all 18 complement titrations where the titer was 1:50-1:100 plus, an arbitrary dose of 1 cc. of 1:43 was used in the tests as recommended by Kolmer.

After the duplicate titrations were run, the routine blood serologies were run in duplicate, according to the simplified test of Kolmer using Kolmer salt and Kolmer salt plus $CaCl_2$. In all, 1338 sera and 16 spinal fluids were tested.

The results of the two procedures were in complete agreement in 1290 sera and 16 cerebrospinal fluids. The results disagreed in 48 sera. (See Table 2.)

Table 2

COMPLETE AGREEMENT (Kolmer salt and Kolmer salt plus calcium)			DISAGREEMENT (Kolmer salt and Kolmer salt plus calcium)
	Blood sera	Spinal fluid	Blood sera
Negative	1236	15	
Positive	49	1	
Doubtful	5	0	
			48

Of the 48 disagreements (Table 3) only 9 showed weakly reactive or reactive VDRL's. One case was a syphilitic under treatment which showed negative Kolmer, reactive VDRL, and 2 plus reaction with $CaCl_2$. Another a syphilitic under treatment showed negative Kolmer, negative VDRL, 4 plus with $CaCl_2$. In only one case was the Kolmer alone more reactive than the Kolmer plus $CaCl_2$. This was an unbilical cord blood serum from

a previously treated mother. Further analysis of the clinical evidence of disease was not attempted.

Table 3
Analysis of the Disagreements

No. of Tests	With $CaCl_2$ Salt	With Kolmer Salt Alone	VDRL
5	4 plus	neg.	Non reactive
2	4 plus	3 plus	Non reactive
4	4 plus	2 plus	1 non reactive 3 variable
5	4 plus	1 plus	4 non reactive 2 variable
1	4 plus	plus Minus	non reactive
1	4 plus	Anti-c	non reactive
3	3 plus	neg.	2 non reactive 1 reactive
1	neg.	3 plus	1 weakly reactive
1	3 plus	1 plus	non reactive
6	2 plus	neg.	5 non reactive 1 reactive
2	2 plus	1 plus	1 non reactive 1 weakly reactive
13	1 plus	neg.	non reactive
2	Anti-c	neg.	non reactive
1	Anti-c	4 plus	non reactive
1	Anti-c	3 plus	non reactive

Summary of Results

1. With one exception, there was no difference in the speed with which the antigen, hemolysin or serum controls became clear between those set up in Kolmer salt alone and those set up in Kolmer salt plus calcium chloride.

2. As there were very few anticomplementary sera, any disagreement between the two series are not statistically significant.

3. The addition of calcium salt enhanced the titer of the hemolysin from (1 to 3 tubes) or 1:500 to 1:3000 dilution in 17 out of 19 instances.

4. In all but one titration the titer of the complement was markedly increased using calcium salt. The increase was from 1:50 to 1:100 plus or from 2 to 7 tubes.

5. In a total of 1338 sera, there were 48 discrepant results which is 3.5% of the total sera tested.

Discussion

From the results I would conclude that:

1. The addition of calcium chloride to Kolmer salt:
   (a) increases the titer of the hemolysin slightly
   (b) increases the titer of the complement significantly.

If, however, one must use an arbitrary dose of 1 cc. of 1:43 of complement as recommended by Kolmer, little is to be gained by the increased titer. If one may use the complement in its maximum titer, then a distinct saving in the amount and hence cost of complement would be realized.

2. Using the arbitrary dose of 1 cc. of 1:43 complement in the

comparative tests, the addition of calcium chloride to the Kolmer salt increased the possible false positive reactions in 44 cases. This is 3.2% of the sera tested.

(Acknowledgment is made to Sharpe & Dohme for their free contribution of all the complement used in doing these tests and their instigation of the work. Also to Dr. John W. Eiman for his helpful criticism of the paper.)

REFERENCES

1. Browne, Alcore S. et al. Am. J. of Clin. Path., Vol. 24, No. 8, Aug., 1954, 934-945.
2. Kolmer, John A., Spaulding, E. H., and Robinson, H. W. Appleton Century Crofts Co., Inc., New York, 5th ed., 1951.
3. Kolmer, John A., and Elsa R. Lynch, MT (ASCP). Am. J. of Clin. Path., Vol. 24, No. 8, Aug., 1954, 946-953
4. Stackhous, Eleanor A., MT (ASCP). Am. J. of Med. Tech., Vol. 12, No 6, Nov., 1946.

AMONG THE NEW BOOKS

INTRODUCTION TO VIROLOGY: by Gilbert Dalldorf, M.D., Director, Division of Laboratories and Research, N. Y. State Dept. of Health, Albany, N. Y. Charles C. Thomas, Springfield, Ill. 1955. 102 pages. 6 Illustrations. 4 Tables. $3.50.

As its title implies, this volume serves to acquaint the physician and microbiologist with the basic principles involved in virus diagnostic techniques. The first eight chapters cover the classification, diagnosis, pathogenesis and immunology of the virus diseases most common to America. Chapter nine is devoted to the planning of a virus laboratory and ten to "The Simple Common Technics." These last include a brief resumé of the preparation of antisera, animal and egg inoculations, as well as tissue culture and serological tests.

MEDICAL AND PUBLIC HEALTH LABORATORY METHODS: (6th Edition), edited by James S. Simmons, S.B., M.D., Ph.D., D.P.H., Sc.D. (Hon) Late Brigadier General, U. S. Army (Retired); Dean and Professor of Public Health, Harvard School of Public Health; Former Chief, Preventive Medicine Service, Office of the Surgeon General, U. S. Army. And Cleon J. Gentzkow, M.D., Ph.D., Colonel, U. S. Army (Retired); Director, Bureau of Laboratories, Department of Health, Commonwealth of Pennsylvania; Former Chief; Division of Chemistry and Physics, Army Medical School, Washington, D. C. Lea & Febiger, Philadelphia 6, Pennsylvania. 1955. 1191 pages, 115 illustrations, 9 color plates, 129 tables. $18.50.

This volume represents the cooperative efforts of 35 authorities who have recorded their experiences in handling thousands of specimens under conditions enabling complete control. It has been developed as a comprehensive working manual and reference book for clinical and public health laboratories, and as a textbook for use in schools of medicine and of medical technology. There is an excellent balance of theory and practice of the various methods described.

All chapters of this edition have been rewritten completely with the exception of those on Statistical Methods. Many recent scientific advances, new technics and instrumentation, are included. Some have not been published previously.

Among the new and revised subjects are fluorometry, flame photometry, paper chromatography, liver function tests and their characteristic behavior in patients suffering from liver disease, true reducing sugars in blood and urine, rapid turbidimetric method for determining amylase of serum, for isolation and identification of poisons in insecticides and rodenticides. There is a larger section on mycology, rickettsiae and viruses, with a new chapter on antibiotics, culture media and new methods of bacteria identification, and antigenic analysis for species identification of enteric pathogens.

THE ROLE OF LYMPHOCYTES AND PLASMA CELLS IN THE FORMATION OF ANTIBODIES

VERNETTA HIDDE, M.T. (ASCP)
39 Grand Ave., Billings, Montana

The reticulo-endothelial system had long been considered the site of antibody formation in the body. With new experiments emphasis was shifted to lymphocytes and plasma cells as possible sources of body antibodies.

As early as 1935 McMaster and Hudack[1] concluded that agglutinins were formed within the draining lymph nodes of mice following intradermal injections of antigenic material.

Harris et al[2] conducted a series of studies of popliteal lymph nodes together with the lymph emerging from such nodes before and after injection of antigens into the foot pads of rabbits. Results showed that lymph emerging from such nodes showed antibodies earlier and in higher concentration than the blood stream and that lymphocytes contained in the efferent lymph draining the injected area were richer in antibodies than the lymph plasma surrounding them. Further observations of the popliteal lymph nodes distal to the injection point of the antigen showed the nodes become progressively larger, with the increase in size being largely due to the enlargement of the cortex area of the node. Normal cortex of rabbit nodes showed lymphocytes with only a few plasma cells along the sinuses, while after hyperplasia no plasma cells were noted except in the medulla where they did not appear to have increased over their concentration in uninjected rabbits. Investigating from another angle they demonstrated the presence of ribonucleic acid in cells from such nodal areas and found that its concentration peak occurred at or slightly before the appearance of the maximal concentration of antibodies in the same node. This peak was more than twice the normal value and occurred two to five days following injection of the antigenic material into the foot.

Physico-chemical studies of normal and stimulated lymph nodes and lymphocyte extracts from such nodes conducted by Harris, Moore, and Farber[3] reveal that components with higher mobilities were increased after injection of the antigen, whereas the component with mobility similar to gamma globulin was not significantly increased. Since all antibodies described have mobilities identical with that of serum gamma globulin and, since a marked increase in total protein occurs after stimulation, some other phenomena—other than the production of protein with a mobility of gamma globulin—must be occurring in lymphoid cells of nodes draining the site of antigen injection. The ultracentrifuge revealed only components with sedimentation constants much lower than that of serum gamma globulin in cell

extracts, both before and after stimulation. The authors suggested that the low sedimentation constant of antibody containing lymphocyte extracts indicated that after the injection of antigenic material there appeared in the draining lymph node a significant amount of a substance of either low molecular weight or of low density—such as lipo-protein—which may be nonspecific or may be the precursor of serum antibody.

Some contradictory results have appeared in the work of Craddock, Valentine, and Lawrence,[4] who conducted an experiment with lymphocytes from the thoracic duct of cats and failed to find any indication of the presence of antibodies in washed lymphocytes. Cats exposed to X-ray radiation showed slight or negligible amount of antibody released from lymphoid tissue after X-ray damage of cells. Their conclusion was that there was no evidence of lymphocytic transport of antibody to blood.

Erslev[5] worked with lymphocytes from rabbits hyperimmunized with a polyvalent pneumococcal vaccine and found no significant amount of antibody protein in lymphocytes in spite of a significant amount of antibody in the same volume of plasma.

The effect of anti-lymphocytic serum on antibody levels was investigated by Woodruff, Forman, and Fraser.[6] Previously Dougherty, Chase, and White had reported that if immunized animals were kept until the antibody titer had fallen to a low level and then were injected with adrenal cortical extract a sudden rise in the titer of antibody would occur. This is the so-called anamnaestic response and was attributed to release of antibody from lymphocytes which were considered to be destroyed as a consequence of the injection. This latter explanation was challenged because there was no definite data to indicate that the mechanism of ACTH which resulted in lymphopenia was lysis of lymphocytes. Other workers had found no change in the level of circulating antibodies following adrenal stimulation. So these three workers administered antilymphocytic serum to rats previously immunized with Salmonella paratyphi B with the resultant absence of an anamnaestic response, i.e., failure to produce a rise in flagellar agglutinin titer despite a profound fall in the number of circulating lymphocytes. This fall in lymphocyte count occurs even in adrenalectomized animals, seeming to indicate that the action of the serum is not dependent on adrenal mechanism. Antilymphocytic serum was not well tolerated by adrenalectomized animals—the death of two being reported. The authors reasoned that the lack of anamnaestic response was not surprising in view of the fact that antilymphocytic serum had little or no deleterious effect on suspensions of lymphocytes in vitro.

Harris and Harris[7] think that biological and technical factors may explain these differences; one point being the extreme

fragility of mature lymphocytes. They believed that the washing of lymphocytes must be extremely gentle or washing will destroy the titer. The experiment of Craddock et al[4] they criticized on the grounds that the cell sediment used was from the thoracic duct lymph and this being a central repository for lymph, the lymphocytes therein may have lost their antibody by leakage or rupture into lymph plasma. In order to prove the point that biologic and technical factors were contributory in the experiments investigating antibody formation in popliteal lymph nodes, they used a series of rabbits, some being injected with 0.2 ml. of a suspension of dysentery bacilli and some receiving five times this amount. Injections were made in one hind foot pad. In animals receiving the smaller amount of antigen they reported confirmation of their former results which showed the popliteal lymph node of the injected leg contained relatively more antibody in comparison with the opposite node. With antigenic subcutaneous injections elsewhere in the body there was no difference in antibody content of the two lymph nodes. Larger doses tended to show less difference and they thought this may have accounted for some differences in experimental results.

Some experiments have seemed to establish plasma cells as an agent in antibody formation. Working with the idea that correlation between hyperglobulinemia (due to high concentration of antibody) and an increase in plasma cells (myeloma cell proliferation) was definitely established and with the fact that autopsy animals which had had an established hyperglobulinemia showed plasma cell proliferation in practically all organs, the degree of plasma cell proliferation seemingly proportional to the concentration of antibody protein; Bjornaboe, Gormsen, and Lindquist[8] set up an experiment for further work on plasma cells in antibody producing rolls. Since Fragraeus had found that plasmapheresis in rabbits was not accompanied by an increase in plasma cells, they raised the question if only pathological globulin—especially antibody protein—could be formed by plasma cells. Rabbits were immunized with a mixture of eight pneumococcus types and a massive plasma cell infiltration (90%) and a slight lymphocyte infiltration (10%) occurred in the adipose tissue of the renal sinus. Extracts of this adipose tissue, rich in plasma cells, were found to contain considerable amount of antibody protein, essentially more than was found in extracts of any other organ from these animals. Since lymphocytes were found in the renal sinus fat in comparatively small amounts, and, since extracts of thymus from highly immunized animals contained no larger amount of antibody than did extracts of muscles which they looked upon as almost free from lymphocytes, the authors thought it highly improbable that lymphocytes would give the high antibody protein concentration which extracts of

this tissue produce. Thus they advanced the hypothesis that antibodies are produced by plasma cells.

Supporting this hypothesis, the cytoplasma of plasma cells has been found to possess a high concentration of ribonucleic acid, which is intimately involved in protein production.[9]

Another experiment by Reiss, Mertens, and Ehrich[10] involved agglutination of bacteria by lymphoid cells. It was found that these cells from antibody forming lymph nodes agglutinate on their surface, in vitro, the bacteria with which the animals were immunized. The authors identified some of these cells which showed surface agglutination as belonging to the plasma cell series, whereas typical small lymphocytes failed to show this phenomena. Thus it was concluded that plasma cells, not lymphocytes, elaborate agglutinins.

Splenic cells have been used in attempts to determine the role of plasma cells and lymphocytes in antibody production. Rich, Lewis, and Wintrobe[11] first connected the lymphocyte with the body's reaction to foreign protein when working with acute splenic tumor; they found the conspicuous cells were lymphoid in character.

Keuning and Van Der Slikke[12] worked with rabbits immunized by three intravenous injections of paratyphoid B vaccine. Both red and white pulp were found to produce agglutinins, the red pulp producing more than the white. In the red pulp were characteristic aggregates of immature plasma cells. In splenic cell suspensions separated by sedimentation it was found that both large immature cells and small lymphocytes probably contained agglutinin but only the former were capable of synthesizing antibody.

The great numbers of large, immature cells in the characteristic cell aggregates of the red pulp together with the high agglutinin content found in red pulp led the authors to suggest that antibodies will first be produced in the immature plasma cells of such splenic red pulp. Their conclusion was that plasma cells played an important role in antibody formation.

The white splenic pulp contained no plasma cells but showed immature lymphoid cells and these cells gave apparent production of agglutinin in vitro. Likewise mature lymphocytes contained agglutinin so it was thought that the lymphoblastic cells of Malpighian corpuscles might also be involved in antibody production but this role of lymphoblasts during lymphocytogenesis was still considered to be questionable and the two workers ruled out, to their satisfaction, the idea of antibody production by mature lymphocytes.

Recalling that Rich demonstrated that "the acute splenic tumor cell" occurring in acute infections was identical with the lymphoblast in its amoeboid movements and that Sundberg con-

sidered the reticular lymphocyte as the stem cell for both the lymphocytes and the plasma cell, Keuning and Van Der Slikke suggested a possible relationship between the processes of plasmacytogenesis and lymphocytogenesis.

The results of experiments by Craddock and Lawrence[4] who found that antibody formation was completely impaired if X-ray radiation which destroys lymphopoietic tissue was applied to the whole body previous to antigen administration and that the immunization was not interfered with if the X-ray radiation was delayed until three days after the immunization at a time when antibody production had just started, seemed to point up the fact that lymphopoietic tissue is essential for antibody formation, but this process, once started, goes on notwithstanding destruction of lymphopoietic tissue proper. As an explanation of this phenomena Keuning and Van Der Slikke presented the plasma cellular reaction found in their experiment.

To reconcile their experimental results and those of others, some of which seemed contradictory, two hypothetical possibilities of relationship between lymphocytogenesis and plasmacytogenesis were advanced.

One: Following administration of antigen, lymphoblastic cells during maturation give rise to (1) antibodies and to lymphocytes still containing some antibody and simultaneously (2) to antibodies and to plasma cells containing a large amount of antibody. The antibodies of lymphocytes would be lost rather soon, while those of the plasma cells would be retained longer and would be liberated gradually over a long period.

Two: Lymphocytogenesis as such is not involved in antibody production but that the lymphoid stem cells arising in great quantities during antigen stimulated lymphoporesis are involved by providing cellular material for plasmacytogenesis.

They added further that the mature plasma cells of the Marschalko type, in either case could be expected to play an additional important role—as, for instance, in anamnestic response by providing the cellular material for renewed antibody production.

Thus, in the future, the two cell types, plasma cells and lymphocytes, may be found to perform a definite role in antibody formation or to stem from the same cell type which performs in this role.

BIBLIOGRAPHY

1. McMaster and Hudack. Journal Experimental Medicine, **61**: 783, 1935.
2. Harris and Harris. Journal Experimental Medicine, **90**: 169, 1949.
3. Harris, Moore, Farber. Journal Biological Chemistry, **179**: 369, 1949.
4. Craddock, Valentine and Lawrence. Journ. Lab. & Clin. Med., **34**: 158, 1949.
5. Erslev, Allen. Journal of Immunology, **67**: 3, 281, 1951.
6. Woodruff, Forman, Fraser. Journal of Immunology, **67**: 1, 57, 1951.

7. Harris and Harris. Journal of Immunology, **64**: 45, 1950.
8. Bjrneboe, Gormsen and Lundquist. Journal of Immunology, **55**: 121, 1947.
9. Wintrobe, M. M. Clinical Hematology, p. 196-197, 1951.
10. Reiss, Mertens, Ehrich. Soc. for Exper. Biology and Medicine, **74**: 732, 1950.
11. Wintrobe. Ibid., 9.
12. Keuning, Van Der Slikke. Journal of Laboratory and Clinical Medicine, **36**: 2, 1950.

AMONG THE NEW BOOKS

ELECTROCHEMISTRY IN BIOLOGY AND MEDICINE: Edited by Theodore Shedlovsky. John Wiley and Sons, New York, 1955, 369 pp. $10.50.

This book grew out of a symposium sponsored by the Electrochemical Society in 1953. Written by experts, actively working in the fields they discuss, the book presents the recent advances and current thinking in electrochemical problems of living processes.

Included are discussions of electrocardiography, electroencephalography of brain tumors and epilepsy, as well as ion distribution and its relation to resting and action potentials in plant and animal tissues such as nerves and muscles. The techniques used to study these problems, such as the preparation and study of membranes of predetermined porosity and charge is covered extensively.

This field is relatively new and very active. The contributions in this book are well written and interesting. The book should serve its purpose of stimulating further discussion and research into the chemical mechanisms of electrical activity of living processes.

METHODS OF BIOCHEMICAL ANALYSIS—Vol. II: Edited by David Glick. Interscience Publishers, Inc., New York, New York (1955). $9.50. 470 pp.

This is the second volume in an annual series designed to present authoritative reviews of the progress in biochemical methodology. The series contains recent developments in techniques of interest to laboratories engaged in research in biochemistry and should be a boon to clinical laboratories, which, being largely understaffed, do not have the time to keep up with and evaluate the latest in chemical techniques.

The chapters in this volume which should be of particular interest to those in clinical chemistry are: Chemical Determination of Adrenaline and Noradrenaline in Body Fluids and Tissues by Harold Persky; Lipid Analysis by Warren Sperry; Assay of Proteolytic Enzymes by Neil Davis and Emil Smith; and Determination of Serum Glycoproteins by Richard Winzler.

QUANTITATIVE METHODS IN HISTOLOGY AND MICROSCOPIC HISTOCHEMISTRY: by Oliva Eranko, Docent in Microscopical Anatomy, University of Hersinki, Assistant Director of the Physiological Department, Institute of Occupational Health, Helsinki, in statistical collaboration with Jaakko Kihlberg, Head of the Statistical Section, Institute of Occupational Health, Helsinki. Little, Brown and Company, Boston, 1955. 160 pages. Illustrated. $5.50.

The authors have successfully explored and explained the quantitative aspects of material and methods in histology and histochemistry within their personal experiences, compiling such material in the single volume from many studies that have been in use previously. The book is designed for those histologists and histochemists who are interested in quantitative analyses.

The contents include Variability and Its Measurements, Animal Experiments, Preparation of Microscopical Specimens, Microscopic Demonstrations of Tissue Details, Estimation of Relative Volume, Counting, Sizing and Various Quantitative Criteria, Absorption and Intensity of Light Measurements, and Statistical Analysis of Results.

MEDICAL TECHNOLOGISTS IN CANADA*

D. W. PENNER, M.D.

Pathology Department, Winnipeg General Hospital, Winnipeg, Manitoba

No two countries in the world resemble each other in their type of people and way of life as closely as do Canada and the United States. Yet in each country there are wide variations in both climate and living habits. Life within the Arctic Circle in Northern Canada is rather different from life in Toronto, but such wide variations also exist in the United States. Canadian technologists, their training and working conditions, differ very little from their fellow American technologists.

It might be of some interest to our American friends to review the development of medical technology within Canada and compare it with that in the United States. Recognizing the necessity for a standards body who would set up a uniform standard of training for technologists, the American Society of Clinical Pathologists, some twenty years ago, established a Registry of Technologists in the United States. Under the aegis of the A.S.C.P. rapid advances were made, and in the subsequent years educational standards were gradually raised and examinations conducted semi-annually. The Council on Medical Education and Hospitals of the American Medical Association and the American Hospital Association cooperate in the training program of the American Society of Clinical Pathologists. Through the American Registry and the efforts of the American Society of Medical Technologists recognition of the technologist has been accepted by the authoritative medical bodies.

In Canada as in the United States, similar conditions pertained in the late thirties regarding the status of the technologist. There had been no unified effort either provincially or throughout the Dominion as a whole, and the large group of technicians in Canadian hospital laboratories were without official recognition until 1937, when the Canadian Society of Laboratory Technologists was incorporated under Federal Charter, and later under agreement with the Canadian Medical Association became the official Registry of Technologists throughout Canada. The C.S.L.T. has gradually grown in stature.

Following the advice and counsel of the Special Committee of Pathologists appointed by the Canadian Medical Association, and with the aid and direction of pathologists and laboratory directors in hospitals throughout the country, the educational and technical qualifications of technologists have been raised to a point where certification by registration has become the accepted standard throughout Canadian hospitals.

With the exchange over the 49th parallel of personnel in all fields of endeavour, the equating of standards particularly as applied to academic requirements has become a necessity. The

* Received for publication September, 1955.

American Registry's basic requirement for admission to the training program is two years College with the necessary credit hours in specific science subjects. Our Canadian students are required to have Senior Matriculation which must include Chemistry, one of either Physics or Biology and mathematics. The latter is accepted by the American Registry as equivalent to first year of College in the United States.

The basic minimal period to be spent in a rotational program through the approved schools of the American Registry is one year. The basic minimal period of training for our approved schools of training is also one year, but a large percentage of the approved schools throughout Canada have now increased their training program to eighteen months and two years. Two provinces, namely Alberta and Saskatchewan, have increased their training period in all their approved schools throughout the province to two years, and others are gradually following suit. Interesting test programs are being tried out in several Universities and Junior Colleges in a two-year program aiming towards registration. It consists of a full academic year of first year University credit subjects including organic, inorganic and biochemistry, bacteriology, etc., followed by four months of concentrated practical training at the bench under the supervision of highly qualified instructors in hematology, bacteriology, serology and biochemistry. The student then spends one year in rotation through an approved hospital laboratory. These training methods are being watched with keen interest. University courses leading to a degree also exist in several Universities, some preparing the candidate for the general field and others for the special fields.

Whether the program of training be one wholly of apprenticeship on the Senior Matriculation level or whether it be on the Junior College or University level the student writes the examination for registration at its completion. Successful students receive their certificate of R.T. and automatically become members of the C.S.L.T. Specialist certificates are open to those qualifying by additional training in the six special fields of biochemistry, bacteriology, serology, hematology, histology and blood bank technique. Many hospitals have now established a salary differential for this additional qualification, and our number of specialist certificated members is on the increase.

Refresher work in Canada is as yet at a premium. There are a few short courses of a specialized nature, but most Canadian technicians go to the United States to participate in the variety of programs available there. At the first combined meeting between the American Society of Medical Technologists and the Canadian Society of Laboratory Technologists to be held June 17th to 22nd, inclusive, at the famous Chateau Frontenac in

Quebec City in 1956, we plan to initiate a program of refresher courses in conjunction with the annual convention of both Societies.

The foregoing deals with the professional development and standards in our two countries. Since a large number of Canadian technologists leave Canada to work in the United States, it may be of some interest to speculate as to the reason for this. This might point out some of the differences which may exist. It has been stated many times that the economic opportunities are much better in the States. This may actually be true, but it is difficult to be sure. Canada certainly enjoys a very high standard of living. The basic laws of supply and demand work in Canada as they do in your country. There is a tremendous variation from area to area and from institution to institution. The more isolated, and usually the smaller the hospital, the higher the salary. Rural areas generally command a higher wage than large urban teaching centers. The cost of living varies considerably as well. Although certain differences undoubtedly do exist, these are not nearly as great as the variation within each country.

Climatic and geographic differences between our countries undoubtedly account for some of the migration of our technicians. Your own northern states differ very little from our southern areas. It is generally not realized that the greatest portion of our population is located in relatively small southern areas of Canada. True, even the far northern area is inhabited but so sparsely that there are few technicians employed. The Canadian midwest is characterized by sunny, warm, relatively dry and all too short summers with sunny, cold dry winters. The southern portion of Ontario has a somewhat shorter and less severe winter. Our east and west coastal areas are almost identical with those of your northern coastal states. There are no areas in Canada where snow and below freezing weather do not occur during the winter months, and for the most part winter consists of many months of snow with freezing cold weather. In the Arctic area we have the land of the midnight sun, where for a short period during the summer the sun can be seen throughout the twenty-four hours of the day, and then for a period during the winter the sun never comes above the horizon.

The question of opportunity is another factor to consider. The population of the United States is approximately ten times that of Canada. There are certainly more and larger cities and more and larger medical centers. There are, therefore, more positions available as well as numerically greater chances for advancement. But at the same time there are ten times as many people competing for those opportunities. Both of our countries are economically wealthy, and are growing and developing. There

is no question that Canada has not as yet reached the same degree of development in certain technical and research fields, and there are less funds available for certain types of research. Yet this difference is not marked and in the last ten years there has been a very rapid increase and development of medical research in Canada. Perhaps there is a difference in basic attitudes between hospital, technical staff and management relationships. It is said that in the average American hospital laboratory the relationships between pathologist and medical technologist is more informal than in Canada. But this again varies greatly from area to area within the country. I would think it impossible to be more informal than I have observed in some Canadian laboratories; although I am told that Canadians are much more reserved than their American friends.

There may be very little basic difference in salaries and opportunities between United States and Canada but a large proportion of Canadian technologists migrate to the United States every year, whereas it is my impression that very few American technologists come to Canada. I would think that the main reason for the Canadian migration are those not directly related to their work, but rather the desire to see the country, the attraction of the milder climate, the appeal of certain large American cities, and above all else the fact that distant fields look greener. Perhaps the fact that we Canadians have not "sold" our country to the American half as effectively as they have theirs to us, accounts for the absence of the reverse migration. Perhaps we spend too much time pointing out how we are almost as good and almost as advanced as our American friends and too little time telling ourselves and them that we, our country and technologists, are second to none.

Having worked and visited in a number of Canadian and American hospital laboratories I find it very difficult to point out any real differences. As already stated these differences appear to be greater within each country than between two countries. It is perfectly true that I strongly suspect that no American technician has ever crawled on his hands and knees into a snow and ice igloo at 45° F. below zero to take a blood count from a sick Eskimo as I know one Canadian technician to have done—but this is hardly the average daily experience for Canadian technicians. Nor do our technicians in the outlaying areas have to spend half their time fighting off bears or Indians in their "backwoods hospitals." We do not even have a Canadian Davy Crockett Legend! Since there are only minor differences, and because our problems and ways of life are so similar, our mutual efforts must certainly lead us to the same goal, which is the provision of laboratory aids, to those whose business it is to treat and to prevent sickness.

In 1956 Canadian technologists play host to our American friends. We extend a warm welcome to you—we hope you will come to the meeting and we also hope that you will visit often and learn to know us and the country we live in.

AMONG THE NEW BOOKS

LABORATORY IDENTIFICATION OF PATHOGENIC FUNGI SIMPLIFIED: by Elizabeth L. Hazen, Ph.D., Associate Bacteriologist (Mycology) and Frank Curtis Reed, Laboratory Illustrator and Photographer, Division of Laboratories and Research, New York State Dept. of Health, Albany, N. Y. Charles C. Thomas, Publisher, Springfield, Illinois. 1955. 108 pages. 22 illustrations. $5.50.

Well illustrated, with presentations of the characteristic features upon which is based the identification of pathogenic fungi, this volume should be of much value in any teaching laboratory. Photomicrographs are used, together with a minimum of text giving the cultural and microscopic characteristics. Tables show the media for macroscopic as well as for microscopic identification. There is a list of essential culture media with their formulae, and a selected list of references.

APPLIED MEDICAL BIBLIOGRAPHY FOR STUDENTS: by William Dosite Postell, Medical Librarian and Professor of Medical Bibliography, Louisiana State University School of Medicine, New Orleans, Louisiana. Charles C. Thomas, Publisher, Springfield, Illinois. 1955. 142 pages, illustrated. $4.50.

This volume will assist materially to introduce the bibliographic "know how" to the medical student and others interested in writing or in the use of the medical library. There are three main sections; namely, a brief history, giving the beginnings of Medical literature, followed by a Modern Bibliography with some of the basic reference tools and periodicals, and methods of bibliography, which show the reader many of the principles of bibliographic citations, as well as methods to follow in writing papers. There are appendices listing dictionaries, directories, encyclopedias, indexes, and year books, recommended reading, and study questions.

ALSO RECEIVED

INTRODUCTION TO OPERATING-ROOM TECHNIQUE: by Edna Cornelia Berry, R.N., A.B., Head Nurse, Operating Rooms, University Hospitals of Cleveland, and Mary Louise Kohn, A.B., R.N., M.N. Formerly Instructor in Operating-Room Technique Frances Payne School of Nursing, Western Reserve University, Cleveland, Ohio. The Blakiston Division, McGraw-Hill Book Company, Inc., New York, Toronto, London. 1955. For Ring Binder, 154 pages. $4.00.

INTRODUCTION TO CHEMICAL PHARMACOLOGY: by R. B. Barlow, Research Fellow, Yale University, School of Medicine, Formerly ICI Research Fellow, University of Glasgow, Methnen & Company, Ltd., London & John Wiley and Sons, Inc., New York. 1955. 343 pages, 52 figures. $6.25.

TEACHING PHYSIOLOGY AND ANATOMY IN NURSING: by Hessel H. Flitter, R.N., B.S., M.A., and Harold R. Rowe, R.N., B.S., M.S.; J. B. Lippincott Company, Philadelphia and Montreal. 56 pages. $2.00.

IONIC INTERPRETATION OF DRUG ACTION IN CHEMOTHERAPEUTIC RESEARCH: by A. V. Tolstoouhov, M.D., Ph.D.; Chemical Publishing Company, Inc., 212 Fifth Avenue, New York, N. Y. 276 pages. 12 Figures, 77 tables. $10.00.

LECTURES ON THE SCIENTIFIC BASIS OF MEDICINE. VOLUME III—1953-54. British Postgraduate Medical Federation. John de Groff, Inc., New York, New York. 1955. 21 lectures. 398 pages. 9 plates. $6.00.

THE USE OF A MARGINAL PUNCH CARD IN THE MEDICAL LABORATORY FOR ROUTINE COMBINED REPORTING, FILING, FINANCIAL, ACCOUNTING AND STATISTICAL PURPOSES

ELMER C. SANBORN, B.A., M.T. (ASCP)
Pennock Hospital, Hastings, Michigan

Better ways of doing things in one discipline often go unnoticed in another to the detriment of progress in all fields. Business and accounting have been using the margin punch card for several years. Multiple punch card forms with one or more for each laboratory department (hematology, bacteriology, etc.) are in use in a number of large hospitals,[1] for routine and special applications.

This paper contains a description of the punch card form we designed and the system found useful in a 65-bed hospital. The form is used for combined requesting, recording, reporting, filing and analysis of routine clinical laboratory work. Duplication of data and the saving of time is accomplished by a fine grade carbon applied to the backs of the forms. Marginal punch holes are used on the laboratory copy for organizing and finding data and patient's reports. There are four hospitals here in the Southwestern Michigan Hospital Council area currently using this punch card form, to be described.

Description of the Form

The present system is built around a single form (see Figure 1). This form consists of one set of four sheets gathered on the left margin with carbon applied to the backs of the first three, the fourth is a card stock for filing. For economy and versatility all four are printed from the same plate as shown in Figure 1. The name of the hospital is omitted so that several hospitals may take advantage of quantity purchasing. The first sheet is yellow and is used for sending charges to the business office. The second sheet is white paper and is the patient's chart copy when the form is filled in completely. The third sheet is pink and is the doctor's office copy. These are placed in the doctor's mail box where laboratory results are brought to the doctor's immediate attention. (A very popular and appreciated service.) The fourth stiffer card is the laboratory file copy. Brown ink has been found readable and photogenic for all sheets. Color coding of the paper[2] is to prevent duplication or omission in distribution. It is found that to get clear fourth copies, it is necessary to put the carbon on the backs of the three top sheets. (Additional sheets for disposable carbons make a fourth copy under most pencil or ball point impressions indistinct.) A two-coat "carbonless" type of carbon application[3] is being considered

* Read before ASMT Convention, June, 1955, New Orleans, La.

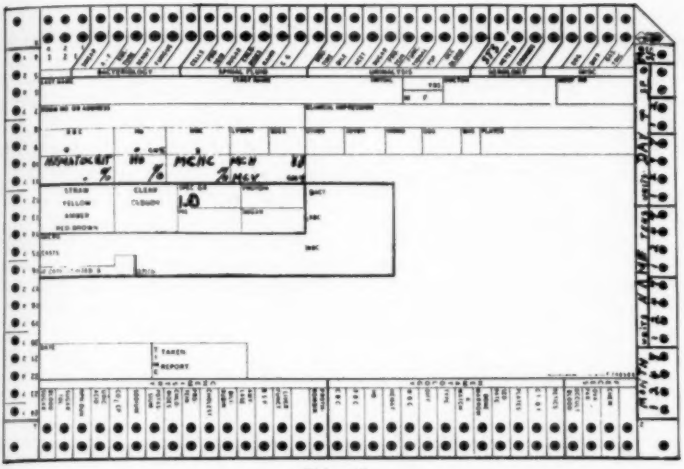

Figure 1
LABORATORY FORM
Original Size 5 x 8 Inches

for the next printing of the form to eliminate the objections of permanent carbon.

It has proven feasible to report all tests on this form. Since most reports are the hematology, urinalysis variety and both requests frequently are made at one time, boxed areas for hematology and urinalysis have been printed on the center area of the form. Other frequently reported tests, serology, type and cross match, prothrombin time, sedimentation rate, etc., can be rubber stamped on as needed in the blank space of the form. This is done by the technologist at the time of receipt of the form in a laboratory or upon completion of collection of the required specimen. Figure 2 illustrates the rubber stamps now in use at this laboratory. These stamps are easily applied to each of the three last sheets of the form. Their printing is superimposed without difficulty, even the decimal points will align.

The center area is obviously a compromise. Those of you who have tried to design the perfect COMPLETE single form will realize it can't be done considering the practical limits of size, personal choice and readability. Some of the large hospitals have solved the problem of limited reporting area by providing different margin punch card sets for each department—bacteriology, tissue, etc., but still employ the quadruplicate form and punch system. Just what size a hospital has to be before it is necessary to have separate forms for each department hasn't

been determined by this author. It was estimated by one doctor[4] as high as 250 beds. In our situation the choice of a single form was influenced considerably by its lower cost. We can justify the cost of one top quality form but not ten different ones. Expense of the forms will be discussed in a later paragraph. The single laboratory form is desirable also from the standpoint of simplicity; sputum culture requests are not mistakenly put on a gastric analysis blank for example. It eliminates making a choice as to what form to use. There is only *one* for *all* laboratory work, which at this laboratory includes EKG and BMR, bacteriology, serology, chemistry, hematology, urinalysis, parasitology, blood bank, but no tissue. For a multiple form punch system which covers this department the reader is referred to the McBee Company.[3] The single form system also takes less desk storage space than that given to the multiple form system.

The rest of the form, the margin punch area and patient identification space will be discussed in the next paragraph.

Uses and Applications of the Form:

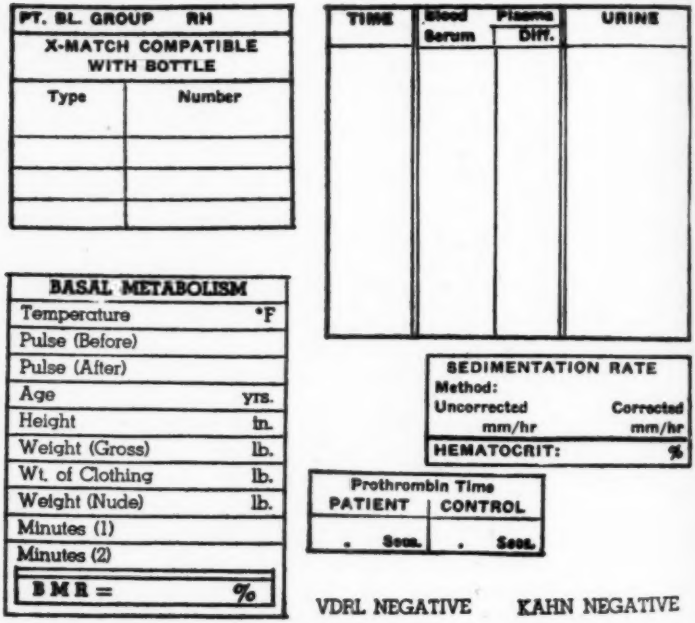

Figure 2
RUBBER STAMP IMPRINTS

A quantity of this quadruplicate form is provided for each of the nursing stations and the laboratory office (for out-patient use). On hospitalized patients, it is nursing's responsibility to fill in the top two lines for identification and laboratory record. She merely circles the test requested on the margin of the card and sees that it reaches the laboratory accompanying any specimen collected by her station or surgery. Catheterized urines are indicated by an "X" in the appropriate box. The recording of the *time* and date of *collection* is important (not the time when the laboratory gets the request or when it's completed, although space for the latter is provided for those who feel it necessary). Thus the form here acts as a request. If the test requested does not appear on the margin, the nurse writes it in the blank space beneath the urinalysis block. The report appears later following and opposite this request. It is desirable to put all tests on one form—the patient isn't then as likely to have unnecessary multiple vena punctures when one will do for collecting both chemistry and hematology specimens.

When the form reaches the LABORATORY, nursing's responsibility ends. Whatever specimens needed are collected by the technologist, and the top (yellow) sheet is torn off, the appropriate charges are written in the blank space and sent to the business office. The business office is thus assured of a copy on *all* work—no missed charges. All the work that the floor requested can be checked against what items the laboratory charged for.

The rest of the form (the back three sheets now) stays with the specimen. Throughout the analysis, the back of the card is used for identification numbers, calculations, recording of the day's standard or control reading, and pertinent remarks for future reference if needed. In our laboratory, blood, and bacteriology smears are given consecutive numbers which are recorded here on the back of the form for future reference to locate a slide when needed. The results of the tests are entered ONCE on the front in the appropriate place. There is no need for ledgers, daily work sheets or copying the patient's name over for any purpose. It is done once by the nurse when she initiates the form. It will be shown later how any information the old day book style record contained can be more accessible in the punched margins of the file card.

At this point in the flow through our laboratory, the report is checked by the senior technologist and/or pathologist for accuracy and appearance (the laboratory report is the only product that leaves the laboratory, it should reflect the care spent in the tests themselves). Was all the work done that was requested? This can be checked as there is no re-copying, you are working with the "request" form.

The second (white) sheet is separated and pigeon-holed to be taken to the patient's chart. "Stat" work is, of course, telephoned, but two deliveries a day to the floor keep most work on time. We prefer to keep the responsibility of putting the report on to the right patient's chart with the laboratory personnel. With this system, copying errors are eliminated and all copies are the same. Scotch tape kept at the nursing station in a dispenser makes quick work of putting the white laboratory report onto the laboratory sheet already in the chart. The succeeding forms are attached one above the other. No data should be covered up by the succeeding entries since the carbon is still present and any writing done above it continues to print through. Also, no separation of the reports are necessary when the charts are microfilmed.

Use of the Form for Outpatients:

The same quad form is used for our outpatients (other hospitals might use it for clinic patients) and work brought in by physicians. The first, yellow sheet is still used for business charges to be turned in by the laboratory secretary to the main business office with cash receipts at the end of the day or is turned in to be billed to the patient or doctor (when he submits the specimen). The secretary fills in the top two lines using a pencil, ball point pen or typewriter. The test requested is circled. She collects the charges using the second white sheet as a RECEIPT to the patient indicating work done and charges (no laboratory results of course). The pink (third) sheet and lab file copy go with the patient into the laboratory where the necessary specimen(s) are collected. These are treated the same as those for inpatients (OPD is indicated in the box, in place of hospital number). The pink copy goes to the doctor's mail box and the file copy is placed in the day's work file together with the inpatient work.

The Laboratory File Copy:

Up to this point in describing the USE of the form we have considered only the aspects of requesting and reporting. Although the margin holes are present on all the forms (it's cheaper to manufacture this way than to punch only the lab file copy) only those in the lab file copy are used. The large hospital's punch card system examined by this author placed their business office copy last. Their cost accounting departments compiled the monthly reports as to number, kind and charges. Their file copies are on a 28 lb. paper or less for filing. In the experience of the present author most small hospital labs make the monthly reports from their records.

If it is desired to have a quad form in which two sheets (i.e., one for business and one for the laboratory) are to be sorted,

one must be 28 lb. weight and the last copy can be the same or preferably on card stock.

At this point in most laboratory filing systems the file copies (often too thin for convenient handling) are arranged alphabetically and filed to be forgotten, unless the original is misplaced and all too frequently this file copy is *also* not available. This latter of course is often lack of attention to detail and system. No form will correct poor work and lack of system. But even if they have been filed correctly by the conventional method only the patient's last name is available. Only by laborious hand sorting of all cards can one get a look at, for example, all the blood sugars on a specific day or over a specific period of time. For that reason most labs also keep daily ledgers, one in each section—chemistry, urinalysis, etc. Some feel the need for a repetitive listing of all serologies and some labs have a ledger for all lab work by *day* and no patient information is available unless one knows the date the work was done! With the punch card system all or any of this information singly or in combination is readily available.

About Punch Cards in General:

The reader familiar with how punch cards may be used may omit this paragraph. There are two kinds of punch cards: those that have perforations in the center of the card and those such as are being considered here, where the operator punches only the margins. The center punch cards (government checks, money orders, are examples) require special equipment for punching, and sorting is done by machine. This type is most useful for large volumes of data and large volumes of cards where machine calculating and printing of the data are desired. The operations possible with margin punch cards are SELECTION AND SORTING (counting is done by hand). No expensive or complicated equipment is needed. The required tools are a needle sorter (a "knitting needle in a handle"), alignment block and hand punch (total cost $11.30).[3]

As will be seen in Figure 1, the form is supplied with holes around the margins.

> "The holes around the sides of the . . . card are coded by notching away that portion of the card between the hole and the edge. These notches allow the coded cards to be separated from unnotched cards when a . . . sorting needle is inserted in one of the holes of a group of cards. Since notched cards have nothing to support them on the sorter, they fall from the group, while unnotched cards remain on the sorter."[5]

See Figure 3.

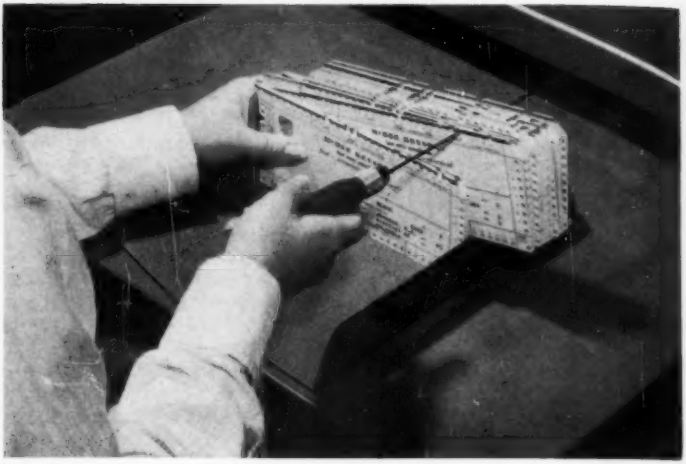

Figure 3
PUNCH CARD SORTING OPERATION

Considering again the flow of the forms through the laboratory, we have arrived at the point where the reports have been made and only the lab report card is left to work with. The next step is punching (coding) the cards. At first, it is recommended to keep the information punched simple and expand as the needs arise. The following information is suggested for a start:

1. alphabetize the last name, 100 category break-down, see Table No. 1.
2. month
3. day
4. outpatient
5. specific tests done.

In our work flow, it is convenient to punch the previous day's cards the first thing each morning.

The double row of holes on the long sides of the form are provided for the specific tests for the purpose of being able to separate the normal values from the abnormal. If the specific test result falls within the normal range only the outer hole is notched away indicating that the test was done and was normal. If the result is above or below the normal range[6] or is abnormal (such as in positive-negative type tests), the *outer* plus the inner hole are notched away.

Table 1—Name Alphabet 100 Divisions

A	1	Co	18	Gree	35	Li	52	Pe	69	St	86
Al	2	Con	19	H	36	Lo	53	Pi	70	Sto	87
Am	3	Cr	20	Har	37	Lu	54	Po	71	Su	88
Ar	4	D	21	He	38	M	55	Pr	72	T	89
B	5	De	22	Hi	39	Mar	56	Q	73	Th	90
Bar	6	Do	23	Ho	40	Mas	57	R	74	To	91
Be	7	Du	24	Hom	41	Mc	58	Re	75	U	92
Ben	8	E	25	Hu	42	Mcl	59	Ri	76	V	93
Bi	9	El	26	I	43	Me	60	Ro	77	W	94
Bo	10	F	27	J	44	Mi	61	Ros	78	We	95
Br	11	Fi	28	Jo	45	Mo	62	S	79	Wh	96
Bro	12	Fl	29	K	46	Mu	63	Sc	80	Wi	97
Bu	13	Fr	30	Ke	47	N	64	Se	81	Wils	98
C	14	G	31	Ki	48	Ni	65	Sh	82	Wo	99
Car	15	Gi	32	Ko	49	O	66	Si	83	X-Y-Z	100
Ch	16	Go	33	L	50	Ol	67	Sm	84		
Cl	17	Gr	34	Le	51	P	68	Sn	85		

The day's stack of cards are then incorporated by sequence sorting of the name codes into the current month's file alphabetically by last name. The specific techniques for coding and sorting are simple. The reader is referred to the *Keysort Punching and Sorting Manual*[5] for the details.

The daily process from the beginning of picking up the stack, punching and sorting to closing the file drawer each day has averaged LESS THAN ONE MINUTE PER CARD.

We keep our current month's cards separate from the year's file and obtain our figures for the number of each test run per month at the end of each month and incorporate the month's cards into their respective places in the year's file. This also is accomplished by using the same alphabetical sequence sorting as for daily sorting. When the file becomes disarranged through use, the restoration of the correct alphabetical sequence can be achieved at any time (it takes about 15 minutes or less to arrange 400 cards).

Let's consider further the operation of SORTING. There are two types—DIRECT and SEQUENCE sorting. Direct sorting is done where cards are dropped by specific holes such as those representing CBC's, blood sugars, etc. Sequence sorting is done to arrange the cards in some desired order within a stack such as alphabetically, chronologically by date, etc.

It is obviously possible to use combinations of the two methods to obtain at any time from the files information such as the following illustrates. When information regarding Kahn serologies is desired, since you have punched these so that the outer hole indicates a negative result and the outer PLUS inner hole a positive result, you can separate negative and positive serologies and arrange them by day and month by sequence sorting. Or, if necessary, one can drop all the Kahns done by any day separately.

Information is thus stored in the punched margins for ready

availability when it is needed.

The holes on the left of the card are numbered serially for reference and also numbered in blocks of 1, 2, 4, 7 for special study purposes, to allow for expansion and versatility. These holes can be used like the top and bottom row holes for single specific information or the information can be coded qualitatively or quantitatively (e.g. ranges of hemoglobin that a patient's test fell in as 11.0 to 11.9 would be punched as one hole, 12.0 to 12.9 as another hole, etc.). Using one group of the 1, 2, 4, 7 to express units, the next group to express tens, etc., any number of "things" or numbers can be coded. (Hole 1 + hole 2 = 3 etc.)

All the possible uses of the punch card have obviously not been explored, and can be adapted to the local needs. The following list will, it is hoped, suggest other uses. All or any one can be used at one time.

Suggested Uses:

1. Obtain data for monthly reports of numbers of each procedure done.
2. Facilitate segregation of data for routine control measures: graph plotting hemoglobin values and hematocrit (or rbc) values with number of these tests done against days of the month, for example, is being kept daily by this and other labs in the area to detect technical and personal errors. Data for this is easily dropped out of the day's stack of cards and recorded. The same data is instantly available for re-check at any later time.
3. Routine or occasional studies can be coded on the side holes:
   a. Number of "stat" orders: punch one hole for "stat" and number code your doctors or floors making each request. At the month's end drop out the "stat" orders and sort them by floor and/or doctors. You then have ammunition to take to your board or administrator when "stat" work becomes disproportionate.
   b. Those procedures which were done during the night, emergency, holiday or week-end tour of duty can similarly be coded routinely or just for a study period for better assignment of personnel to heavy work load time.
4. The study of abnormal values, number of positive enteric cultures, etc., as described above under sorting. It is the author's experience that little or no more time is necessary to punch the normal-abnormal values daily. The information is then always available even if used infrequently. This also adds opportunity to check up on obvious discrepancies in the work which should be re-checked such as a 20,000 wbc and a normal differential (which was missed on the check made at the time the forms were torn apart).

At first this sounds unnecessarily complicated, but in practice, it's logic and usability rapidly improve when you start to work with the cards.

Table 2

Steps	OLD METHODS	Time in Minutes: Sec.	NEW METHOD	Time in Minutes: Secs.
1	REQUESTING—Nurse writes request form	1:00	Nurse initiates lab forms	1:00
2	RECORDING			
	Techn. copies patient data from request			
	a. report form	1:00	Eliminated	0
	b. day book	1:00	Eliminated	0
	c. business office charges	1:00	Write only $ & c	0:10
	Techn. records results of tests:			
	a. report form	0:30	Carbon used for 3 copies	0:30
	b. day book	0:30	Eliminated	0
3	REPORTING			
	Doctor's copy: usually not supplied		Carbon copy	0
	Patient's chart			
	some hosp. re-copy data	1:00	Apply form to patient's chart with scotch tape	:10
	or			
	some paste reports	:10		
4	LAB FILE COPY			
	—record daily no. and kind of procedures	*	Punch and file	1:00
	—daily filing			
	—monthly report		Direct sort, count and record followed by sequence sorting to restore cards alphabetically	:30
	—month's filing			
5	Total time	6:10		3:20

* Record your own figures for these operations and add them to the 6:10 and the new method is still ahead regardless.

Table 3—Relative Costs

OLD		NEW
Form* and daily work books	$0.008	$0.04 in 25M lots** .02 in 100 M lots
Technologist's time†	.206	.103
TOTAL COST for each form in use	.214	.143 maximum .123 minimum

A SAVING OF AT LEAST SEVEN CENTS PER FORM

* Four physicians Record sheets 3 x 5 in. size.

** Group quantity purchasing of the form is economical: four hospitals using 5,000 sets (about a year's supply) will put the cost at about five cents per form. If ten hospitals group together and buy 5,000 sets the cost is only about two cents per form.

† Figures at $2.00/60 min. and time is taken from table 2.

There is much added information available from the punch card system that is hard to put on a dollars and cents basis.

What value can be placed on the means of checking erroneous reports before harm is done? Early detection of a 0.5 Gm.% drop in hemoglobin values can save as much as 25 pints of blood transfused and at $25 per pint is $625. (This occurred at one hospital recently.) This saving is possible through the use of control charts derived from this system of margin punch card.

Economy of Time:

In addition to the time (and thus cost) saved in the REPORTING PHASE which has been considered above, let's consider the time now spent in "book work" for just monthly reports. This author with no previous training of knowledge of the use of punch cards, punched in the 1st 2 months on the average of about 20 cards daily (which happened to be our daily work load for number of *forms*). The following information was punched: name alphabet, date, month, specific tests (abnormal and normal results being separately coded), stat work, week-end work, outpatient work. The time was kept each day by stop-watch and when 999 cards had been punched and filed it was noted that it took 940.5 minutes or 1.06 cards per minute. This was the *total* elapsed time from the picking-up of the stack of each day's cards until these were filed and the drawer closed.

For the month of January the monthly summary (using the form recommended by the American Society of Clinical Pathologists, December 1, 1954) was recorded on separate forms for the In and Out patients and Total procedures for each type of test, and the cards returned to the file drawer alphabetically arranged. The rate for this entire operation of monthly summary (477 cards containing 2167 procedures (no tissue), was TWO CARDS PER MINUTE. Much more information is stored and available than that required by the monthly reports.

Summary

A system and a laboratory-charge-record-analysis-report form incorporating a marginal punch card is described which has been found to be useful, economical and extends the amount of information accessible from clinical laboratory records. This form is at present being used by four hospitals with an average size of fifty beds. It is estimated this single form as illustrated would be useful for 100 to 250 bed size also.

Acknowledgment

The author wishes to thank Vergil N. Slee, M.D., C. Wesley Eisele, M.D. and Margaret E. Waid, M.D. for their contributions to the basic ideas and for their suggestions. The technologists and administrators of the hospitals in Albion, Hastings, Ionia and Marshall, Michigan should be commended for the time, money and suggestions they provided to make this project a success.

FOOTNOTES

1. Miami Valley Hospital, Dayton, Ohio; Youngstown Hospital Association, Youngstown, Ohio, University of Illinois, Hematology Department and others.
2. For 25M set lots. For 100M set lots it is more economical to use the same colored paper and code the ink.
3. McBee Company, 295 Madison Ave., New York 17, N. Y.
4. Personal communication—C. Wesley Eisele, M.D., College of Medicine, University of Colorado.
5. *Keysort Punching and Sorting Manual*, p. 1, McBee Company, NYC.
6. It is suggested for uniformity to use Standard Values in Blood, Albritton, Errett C., W. B. Saunders Company, Philadelphia, 1952.
7. For further description of the use of control charts to prevent errors see the article by Margaret E. Waid, M.D. and R. G. Hoffman, Ph.D., in the Technical Bulletin of the Registry of Medical Technologists, 25: 97-107, April, 1955.

REFERENCES

Albritton, Errett C., *Standard Values in Blood*, W. B. Saunders Co., Philadelphia, 1952.

Kurtz, Dorothy, *Unit Medical Records in Hospital and Clinic*, Columbia University Press, New York, N. Y., 1943.

McBee Company, *Keysort Punching and Sorting Manual*, 295 Madison Ave., New York 17, N. Y.

Waid, M. E., and Hoffman, R. G., The Quality Control of Laboratory Precision Technical Bulletin of the Registry of Medical Technologists, 25: 97-107, April, 1955.

FROM OUR READERS

From D. Whitney, Roselle, Illinois:

"Hint heard at our IMTA workshop on micro techniques at Mt. Sinai Hospital—Use two hemolets together in obtaining blood from an infant's heel—results in greater returns from a double opening with one stab. (It is really excellent)."

BIOLOGICAL HAZARDS OF COMMON LABORATORY PROCEDURES

I. The Pipette

MORTON REITMAN, Ph.D., and G. BRIGGS PHILLIPS, B.S.
Camp Detrick, Frederick, Maryland

Surveys of reported laboratory acquired bacterial infections (Sulkin and Pike, 1951) and viral infections (Sulkin and Pike, 1949) indicate that the number is significant. The laboratory infection may be mild in nature and may even be unrecognized, but all too often serious economic and physical consequences result. Although the means by which the illness is acquired is sometimes unknown, aerosols created by accidents and poor techniques are common causes. Investigations by members of this installation and others have been directed toward analyzing specific bacteriological procedures and equipment, evaluating the hazards involved, and recommending safer practices and procedures.

The information contained in this and subsequent papers represents an attempt to summarize the information available regarding the hazards of common bacteriological procedures. Pertinent references are given.

Methods

The evaluation of most procedures was done by employing nonpathogenic microorganisms and sampling the air by means of sieve type air samplers (duBuy and Crisp, 1944) (Figure 1) during repeated performance of the procedures (Reitman *et al.*, 1953). This sampler has an efficiency between 43 and 73 per cent. Air drawn in

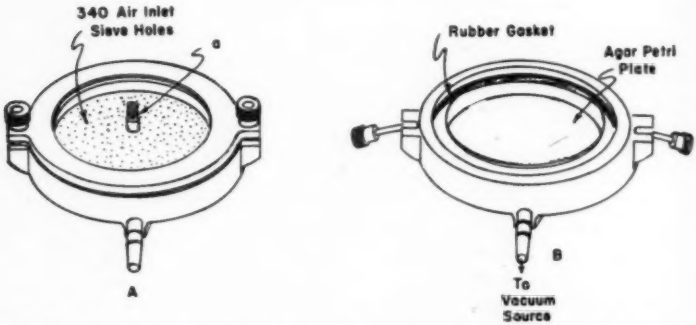

Figure 1—Sieve type air sampler. A. Assembled; a, gage used to measure clearance of sieve top above agar. B. Top removed showing agar plate in position. Air is drawn through inlet holes at high velocity, impinging organisms on agar surface. Sampler operated at rate of 1 cfm.

through the 340 sieve holes is impinged against the surface of an agar plate. Microorganism-bearing particles are deposited on the agar surface and upon subsequent incubation colonies are formed.

Serratia indica, a red pigmented water and soil organism, was used as the test organism. The bacteriological manipulation to be tested was repeated at least 10 times while each of 7 air samplers was operated at the rate of 1 cubic foot per minute around the work area (Figure 2). In each instance, appropriate control sampling revealed none of the test organisms in the air before the test. Serological pipettes were used throughout, since they are the most common in the bacteriological laboratory.

Results

The results summarized in Table 1 are primarily those reported previously by Anderson *et al.* (1952) and Wedum (1953). Although results are recorded as the number of colonies scored after incubation, the number of organisms collected from the air is believed to be greater by an unknown factor, inasmuch as examination of the particle sizes revealed by high speed photography (Johansson and Ferris 1946) make it seem unlikely that all colony-initiating particles contained a single viable organism.

Table 1

Hazards of Pipetting Procedures

TECHNIQUE	Average Number of Clumps of Organisms Recovered from Air During Technique
1. Transfer of liquid cultures:	
(1) Pipetting 2 drops culture into 10 ml broth in 50 ml tube	0.02
(2) Pipetting 2 ml culture into 100 ml broth in 500 ml Erlenmeyer Flask	0.06
(3) Pipetting 2 ml of dilute culture (1:10) into 100 ml broth in 500 ml tube	0.0
(4) Pipetting 10 ml culture into 50 ml tube	0.6
(5) Pipetting 30 ml culture into 50 ml tube	1.2
(6) Pipetting 1 ml culture into 50 ml broth in 125 ml Erlenmeyer flask	1.2
(7) Pipetting 10 ml culture into 1000 ml broth in 2000 ml Erlenmeyer flask	2.4
2. Drop of culture falling 12 inches onto	
(1) Stainless steel	49.0
(2) Painted wood	43.0
(3) Dry hand towel	28.0
(4) Kemrock	23.0
(5) Hand towel wet with 5 per cent phenol	4.0
(6) Dry wrapping paper	3.0
(7) Dry paper towel	1.0
(8) Pan of 5 per cent phenol	0.1
3. Pipette mixing:	
(1) Mixing 7 ml broth culture by alternate suction and blowing, forming bubbles.	0.8
(2) Mixing as above, without forming bubble	0.2
(3) Mixing by suction and slow flow of return liquid down the side of tube	0.0
(4) Mixing by magnetic mixer and then removing of stopper	0.0
(5) Mixing by hand roll, forming bubbles, and then removing stopper.	4.0
(6) Resuspending centrifuged cell in 50 ml tube with 30 ml saline by alternate sucking and blowing.	4.5
4. Blowing out Last Drop from Pipette:	
(1) Blowing out last drop gently, bubbles formed	3.8
(2) **Not** blowing out last drop, no bubbles formed	1.3

Discussion

Transfer of liquid cultures—Probably the most common use of the pipette is to transfer an inoculum to a tube, a flask or a petri

plate. In Table 1, it is clear that aerosols are produced when liquid cultures are transferred by pipette and that the amount of aerosol increases with the size of the inoculum and container. The culture used was a 24-hour broth growth of *S. indica* containing approximately $1x10^9$ cells per ml. It is significant that in one case, item 1 (3) in Table 1, aerosols were eliminated by diluting the culture one log.

Accidental dropping of culture from a pipette—A common accident when using pipettes is the accidental escape of one or more drops which fall to the laboratory table top or floor. The amount of aerosol produced will vary with the height of drop, the type of surface the drop falls upon and other factors. In tests with *S. indica*, stainless steel and painted wood surfaces gave the highest aerosol production but no surface completely prevented aerosol production. The use of a linen towel soaked with 5 per cent phenol or other suitable disinfectant appears most suitable for table tops during hazardous operations.

Mixing culture with a pipette—The technique used in many laboratories for mixing a bacterial suspension in a test tube is that of alternately sucking and blowing with a pipette. If the procedure is to blow in such a manner that air is passed through the suspension, the condition is obviously more hazardous than if care is taken to produce no bubbles. In addition, the procedure is further improved if, instead of blowing, the liquid is allowed to flow slowly down the side of the tube.

When a large number of tubes are involved, a useful device is the magnetic test tube mixer which accomplishes homogeneous mixing with the cotton plug or stopper in place (Phillips *et al.*, 1955). When tubes were mixed by rolling between the hands, bubbles were formed at the surface and aerosols were released when the stoppers were removed.

Blowing out last drop from a pipette—Photographic evidence that aerosols are produced when the last drop is blown from a pipette has been obtained by Johansson and Ferris (1946). This procedure is common in placing an inoculum in a petri dish. When pipetting broth cultures, it is difficult to blow out the last drop without forming bubbles at the tip of the pipette and an aerosol generally results. By *not* blowing out the last drop aliquots can usually be delivered without forming bubbles and the resulting aerosol is less.

Standard TD serological pipettes such as were used in these studies usually have a frosted band around the top indicating that the residual liquid in the tip should be blown out. Snyder (1947), avoided blowing out 1-ml serological pipettes in one technic by delivering 0.9 ml aliquots into petri plates in the preparation of plate counts. Davis and Iverson (1953) determined the possible error introduced by *not* blowing out 1-ml TD, serological pipettes. The average volume of distilled water delivered by 10 randomly selected pipettes when *blown out* was 0.9814 ml. When the same pipettes were

not blown out the average was 0.9636 ml. Thus, the volume of distilled water delivered was decreased 1.78 per cent by not blowing out the last drop.

Oral pipetting—Although pipettes used for bacteriological procedures should always be plugged with cotton in the proximal end, mouth pipetting of infectious materials is unsafe. Even though a cotton plug is used, finger contamination of the end of the pipette may cause oral contamination. The best procedure is to eliminate all oral pipetting by the use of one of a variety of pipettors (Wedum 1950). A bulb device called the Propipette* has been found to work satisfactorily for many operations.

Disinfection of pipettes—After use with highly infectious materials, contaminated pipettes should be placed in a container filled with disinfectant and autoclaved. A flat discard pan is considered safer than the conventional cylinder. The latter provides an opportunity for contamination of the rim of the cylinder and of the top of the pipettes protruding above the level of the disinfectant. In addition, too often the procedure is to remove the pipettes to another

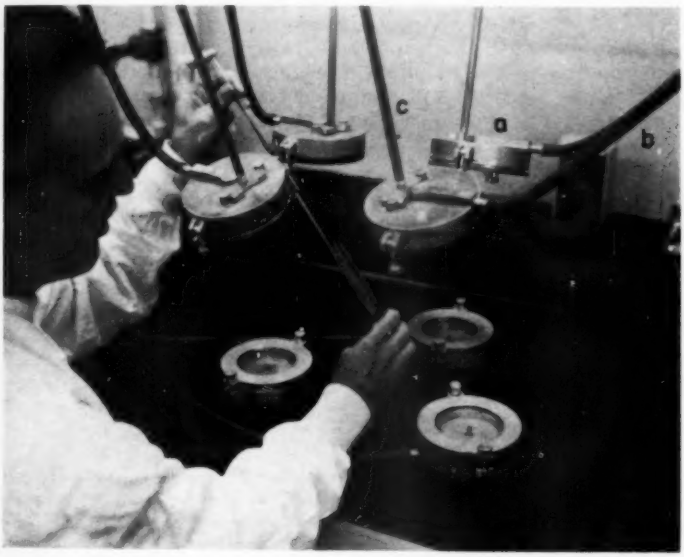

Figure 2—Typical arrangement of air samplers around work area. a. Air sampler, b. vacuum source, c. adjustable sampler holder attached to telescopic frame (not shown).

* Instrumentation Associates, 138 Haven Avenue, New York 32, New York.

container for autoclaving.

Safety rules for use of the pipette—In summarization, the following list of rules is recommended to govern the use of pipettes with highly infectious fluids:

1. If possible, enclose pipetting operations in a ventilated safety cabinet or hood (Wedum 1953) (Phillips, Novak and Alg 1955).
2. Avoid oral pipetting of dangerous materials—use a pipettor.
3. Do not blow out the last drop from pipettes.
4. Do not mix dilutions by blowing air through the pipette into the culture.
5. Place a disinfectant soaked towel on the working surface and autoclave the towel after use.
6. Use flat pans containing disinfectant for the discard of pipettes. Autoclave pan and pipettes together after use.
7. Know the extent of the potential hazards and use appropriate safety measures. If in doubt, do it the safe way.

Summary

The pipette, as it is used in the bacteriological laboratory, can be the source of infection of laboratory workers, if safety measures are not taken which are appropriate to the infectiousness of the microorganisms involved. Particularly to be avoided are the practices of oral pipetting, blowing out the last drop, mixing cultures by alternate suction and blowing through a pipette and accidental drops falling on the bench top.

REFERENCES

1. Anderson, R. E., Stein, L., Moss, M. L., and Gross, N. H., Potential infectious hazards of common bacteriological techniques. J. Bact. **64:** 473-481, 1952.
2. Davis, H. A. and Iverson, W. I. Unpublished results, 1953.
3. Johansson, R. R. and Ferris, D. H. Photograph of airborne particles during bacteriological plating operations. New England J. Inf. Dis. **78:** 238-252, 1946.
4. Phillips, G. B., Hanel, E. Jr., Novak, F. E. and Miller, L. F. Magnetic mixing of test tube dilutions, Applied Microbiol, **3:** 258-263, 1955.
5. Phillips, G. B., Novak, F. E. and Alg, R. L. Portable inexpensive plastic safety hood for bacteriologists. Applied Microbiol. **3:** 216-217, 1955.
6. Reitman, M., Frank, M. A. Sr., Alg, R., and Wedum, A. G., Infectious hazards of the high speed blendor and their elimination by a new design. Applied Microbiol, **1:** 14-17, 1953.
7. Snyder, T. L. The relative errors of bacteriological plate-counting methods, J. Bact. **54:** 641-654, 1947.
8. Sulkin, S. E., and Pike, R. M., Viral infections contracted in the laboratory. New England J. Med., **214:** 205-213, 1949.
9. Sulkin, S. E. and Pike, R. M. Survey of laboratory-acquired infections. Am. J. Pub. Health **41:** 769-781, 1951.
10. Wedum, A. G. Non-automatic pipetting devices for the microbiologic laboratory. J. Lab. and Clin. Med. **35:** 648-651, 1950.
11. Wedum, A. G. Bacteriological safety. Am. J. Public Health, **43:** 1428-1437, 1953.
12. du Buy, H. G., and Crisp, L. R. A sieve device for sampling airborne microorganisms. Pub. Health Rep., **59:** 829-832, 1944.

BIOLOGICAL HAZARDS OF COMMON LABORATORY PROCEDURES

II. The Hypodermic Syringe and Needle

EVERETT HANEL, JR., B.S. and ROBERT L. ALG, B.S.
Camp Detrick, Frederick, Maryland

In a previous paper (Reitman and Phillips, 1955) experimental results were presented showing the hazards involved in the use of the pipette. In this article, the hazards connected with the use of the hypodermic syringe and needle are considered.

The information is principally a summary of previously published studies by Anderson *et al.* (1952), Reitman *et al.* (1954 a.b.) and Wedum (1953) with added recent information.

Methods

Sieve samplers were used as previously described for sampling air around a procedure being tested. Surfaces were examined for bacteriological contamination by means of cotton swabs. Test organisms were *Serratia indica* and coliphage T-3, a bacterial virus. The results represent the general types of procedures in which the needle and syringe may be employed with infectious microorganisms. Studies with coliphage are representative of operations with human or animal viruses.

Results

Studies using *S. indica* as the test organism are summarized in Table 1. These results are self-evident and illustrate the hazardous nature of many operations with the hypodermic syringe and needle. Other results and considerations are as follows:

Finger contamination when using syringes—Fingers or hands may become contaminated two ways; (1) from the syringe plunger and (2) from wads of cotton held in the fingers and used to receive excess fluid and air bubbles from the syringe. Contamination from the syringe plunger usually occurs when the same syringe is used to inject several animals. The rear portion of the plunger becomes contaminated by capillary action and organisms are transferred to the fingers. This can readily be demonstrated by the use of a dye solution in the syringe. The hazard may be minimized by grinding down the rear portion of the barrel, thereby eliminating capillary action.

When it is necessary to expel infectious liquids and air bubbles into wads of cotton, finger and hand contamination can not be eliminated successfully. Tests have shown that a relatively large wad of cotton soaked in a disinfectant should be used. Because finger contamination is unavoidable during this procedure, rubber gloves must be worn.

Withdrawing the needle from a rubber stopper—In addition to the data shown in Table 1, high-speed photographs have revealed that the needle will frequently vibrate and create an aerosol as it is pulled from a vaccine bottle stopper.

Table 1

Use of Syringe and Needle in Bacteriological Procedures

TECHNIQUE	Average Number of Clumps of Organisms Recovered from Air During Technique
1. Hypodermic syringe and needle used with culture in cotton plugged test tubes.	
(1) Drawing inoculum into syringe	0
(2) Expelling inoculum back into test tube (side of tube)	0.2–0.8
(3) Expelling inoculum back into test tube (under liquid)	0.1
2. Hypodermic syringe and needle used to withdraw 1 ml of culture from rubber stoppered vaccine bottles:	
(1) Needle and stopper wrapped in Ceepryn soaked cotton pledget:	
(a) Positive or negative pressure in bottle	0.8–16.0
(b) Atmospheric pressure in bottle or no adjustment	0. – 6.4
(2) Needle and stopper wrapped in alcohol soaked cotton pledget:	
(a) Positive or negative pressure in bottle	0.6–0.5
(b) No pressure adjustment	0.1–0.2
(3) Needle and stopper not wrapped in pledget:	
(a) Positive or negative pressure in bottle	4.4–28.0
(b) Atmospheric pressure in bottle	0.6–19.0
3. Animal injections:	
(1) 10 shaved guinea pigs injected IP with 0.5 ml culture, no disinfectant	16
(2) Same as (1) except unshaven guinea pigs	15
(3) Same as (1) with injection site swabbed, before and after with 1 per cent tincture of iodine	0
(4) Aerosols from closed cage in 1 hour when cage contained:	
(a) 5 guinea pigs injected IP at unshaven sites, no disinfectants used.	11
(b) 5 guinea pigs injected IP at shaven sites, 1 per cent iodine applied before and after injection.	8

Animal injections—Table 1 shows that animal injection sites must be disinfected before and after inoculation to prevent aerosol production and that even when this is done there may be a few airborne organisms recovered in a closed cage following injection. Swabs taken from shaved intraperitoneal injection sites, when 1 per cent iodine was and was not used, further revealed that all sites were contaminated when no disinfectant was used and that 3 out of 10 were contaminated when iodine was applied. Such contamination is attributed to leakage of organisms from the injection site. The use of disinfectant was more important than shaving.

Accidental discharge of liquid from a syringe and needle—An extremely dangerous situation is created when the needle of a syringe containing infectious material flies off and liquid is accidentally sprayed into the air. Simulated accidents of this type have been performed with *S. indica* in a 1785 cubic foot room. When 0.5 ml of culture was discharged into the air and the room ventilation was *off*, 10-minute sieve air samples collected an average of 235 colonies. If the room was being ventilated at the rate of 11 changes of air per hour a fewer number of organisms were collected in the first 10 minutes (124 colonies per plate) but there was wider dispersion of bacterial aerosol in the room.

Most accidents of this type are caused by loose needles. This hazard may be minimized by using only Luer-Lok type syringes. Tuberculin Luer-Lok syringes are now available.

Table 2
Use of Syringe and Needle in Virology Procedures

TECHNIQUE	Viral Particles Recovered in Air Per Operation
1. Using syringe and needle and vaccine bottles to make 10-fold dilutions:	
(1) No cotton around needle	2.3
(2) Ethanol cotton pledget around needle	0
2. Using syringe and needle for intranasal inoculation of mice, number of particles inoculated:	
(1) 1.5×10^8 into each of 10 mice	27
(2) 1.5×10^7 into each of 10 mice	19.9
(3) 1.5×10^6 into each of 10 mice	2.4
(4) 1.5×10^5 into each of 10 mice	0.1
3. Using syringe and needle for intracerebral inoculation of 10 mice with 3.3×10^7 phage particles:	
(1) Inoculation site swabbed with 70 per cent ethanol before	1.1
(2) Inoculation site swabbed with 70 per cent ethanol before and after and needle surrounded with ethanol soaked cotton	0.2
(3) As (2) except site swabbed with 2 per cent tincture of iodine after inoculation	0.3
4. Using syringe and needle for harvesting of allantoic fluid from 5 eggs (avg. count—11.1×10^7 phage particles per ml).	**5.6**
5. Using syringe and needle for harvesting of amniotic fluid from 5 eggs (avg. count—23.3×10^7 phage particles per ml.)	**1.0**

Reconstituting lyophilized culture—In a recent publication on the hazards of handling lyophilized cultures (Reitman *et al.* 1954 a) it was demonstrated that the use of the syringe and needle to reconstitute and transfer lyophilized cultures of *S. indica* usually created detectable aerosols unless the organisms had been lyophilized in a menstruum of 10 per cent milk and 2 per cent glycerin. Other lyophilizing fluids in order of choice were; sheep serum, milk plus broth, nutrient broth and Naylor's solution.

Viral techniques—The hypodermic syringe and needle are widely used in virology procedures. Many of the hazards involved were recently reported by Reitman *et al.* (1954 b). Coliphage T-3 was used to simulate infectious virus particles.

Results are summarized in Table 2. Intranasal inoculation of mice with virus particles appeared to be the most hazardous procedure. During the inoculation, virus particles were recovered by swab from the hands and from the table top. Intracerebral inoculations seem less hazardous; surface samples from the injection site, technicians' hands and table top showed fewer organisms when the method listed in item 3 (3) in Table 2 was used. Although aerosol recoveries while harvesting allantoic and amniotic fluids from eggs showed moderately few viruses, the egg tray and egg shells invariably became highly contaminated and the technicians' hands were contaminated in more than 50 per cent of the tests.

These tests with coliphage demonstrate some of the common uses of the syringe and needle which created hazardous conditions. It was shown that the use of special precautions and careful technic could reduce the hazard but could not eliminate it completely.

Recommendations

Rules Governing the Use of the Hypodermic Syringe and Needle:

1. If possible, when working with infectious microorganisms, use the syringe and needle only in a ventilated safety cabinet or hood.

2. Use only Luer-Lok type syringes.

3. Wear surgical or other type rubber gloves when using the syringe and needle.

4. When removing aliquots through vaccine bottle stoppers, wrap the stopper and needle in a cotton pledget soaked in 70 per cent alcohol or in a proper disinfectant.

5. Expel excess liquid and bubbles into large cotton pledgets soaked in a *proper disinfectant.*

6. Discard syringes into a pan of disinfectant without removing the needle or squirting out the residual culture.

7. Before and after injection of an animal, swab the site of injection with a disinfectant.

Summary

The use of the hypodermic syringe and needle for injecting animals, removing samples from vaccine bottles, harvesting amniotic and allantoic fluid from embryonated eggs, and transferring cultures was found to produce aerosols and surface contamination. Appropriate recommendations for the safety of the laboratory worker are offered:

REFERENCES

1. Reitman, M., Moss, M. L., Harstad, J. B., Alg, R. L. and Gross, N. H. Potential infectious hazards of laboratory techniques; II The handling of lyophilized culture. J. Bact. **68**, 545-548, 1954a.
2. Reitman, R., Alg, R. L., Miller, W. S. and Gross, N. H. Potential infection hazards of laboratory techniques; III Viral techniques. J. Bact. **68**, 549-554, 1954b.
3. Reitman, M. and Phillips, G. B. Biological hazards of common laboratory procedures; I The pipette. Am. J. Med. Tech. **21**, 338-342, 1955.
4. Anderson, R. E., Stein, L., Moss, M. L. and Gross, N. H. Potential infectious hazards of common bacterialogical techniques. J. Bact. **64**, 473-381, 1952.
5. Wedum, A. G. Bacteriological Safety. Am. J. Pub. Health, **43**, 1428-1437, 1953.

ABSTRACTS

HEMOPHILUS INFLUENZAE TYPE B PNEUMONIA

William L. Nyhan, et al. (Yale University School of Medicine) Pediatrics 16, 31-42 (1955).

Three infants, 1 five-year-old boy and 1 sixty-two-year-old man with pneumonia caused by H. influenzae Type b are reported. The organisms grew in pure culture from the blood in 4 and from the lung at autopsy in 1. The value of routine admission blood cultures is emphasized.

DETECTION OF A NEW SEROTYPE OF ESCHERICHIA COLI, E. COLI 0127: B8, ASSOCIATED WITH ACUTE DIARRHEA IN INFANTS

Merlin L. Cooper, et al. (University of Cincinnati) J. Bact. 69, 689-94 (1955).

Bacteriological studies made during an epidemic of diarrhea among infants on two wards of Children's Hospital revealed a new serotype of E. coli, E. coli 0127:B8.

Stool specimens were obtained by using rectal swabs which were placed immediately in test tubes containing 2.0 ml. of sterile glycerin preservative solution (Connecticut State Department of Health, 1945). Cultures were made on sorbitol agar, MacConkey agar and blood agar prepared with trypticase soy agar plus 5% fresh defibrinated rabbit blood.

In addition the swabs were cultured for Shigella and Salmonella on SS agar, MacConkey agar, brilliant green agar and in tetrathionate broth base which, after incubation overnight, was subcultured onto the other three media.

From the acutely ill patients, a predominance of a type of E. coli differing from the usual E. coli was found. They failed to ferment sorbitol, were weak producers of indole, produced colonies on sorbitol agar resembling colonies of E. coli 0111 and 055, but were not agglutinated in the antisera specific for these two types of pathogenic E. coli.

This strain of E. coli fermented sorbitol late, after 13-25 days or not at all. Usual E. coli found in stool cultures produce red colonies on this medium after 18 to 24 hours of incubation at 37° C. while strains of E. coli 0111, 055, and 0127 produce colorless colonies similar to those of lactose nonfermenters on MacConkey agar.

Non-fermenting colonies on sorbitol agar were picked and inoculated into tubes of sorbitol iron agar making stab and surface inoculations. After 18 to 24 hours of incubation at 37° C, the growth from sorbitol nonfermenting colonies either failed to change the appearance of the sorbitol iron agar medium or produced a slight decolorization in the butt of the tube while sorbitol fermenting colonies produced acid, or acid and gas in the butt. It is possible that colonies of other enteric species which do not ferment sorbitol rapidly may be picked. A few colonies of sorbitol nonfermenting microorganisms were subsequently identified as Shigella, Salmonella, or Paracolon.

The strains of E. coli 0127 were nonmotile, methyl red positive, Voges-Proskauer negative, reduced nitrates to nitrites, and produced indole slowly, giving a weakly positive reaction in 3 days and a good positive reaction in 6 or 7 days. Gelatin was not liquefied in 30 days and citrate was not utilized. Purple milk was acidified in 24 hours and coagulated in 3 to 5 days. Growth in triple sugar iron agar produced acid in the slant and acid and gas in the butt without H_2S production. Lactose, glucose, maltose, mannitol, xylose, rhammnose, and arabinose, phenol red carbohydrate broths were fermented overnight with the production of acid and gas. Acid developed after overnight incubation in saccharose raffinose, and sorbose broths, and a small amount of gas developed slowly on further incubation. Dulcitol was fermented after 3 days with production of acid and gas. Salicin and inositol were not fermented in 30 days. Urease was not produced.

The details of the serological studies is presented. All strains were tested by the disk method for sensitivity and were found to be sensitive to neomycin, aureomycin, chloramphenicol, and terramycin and resistant to streptomycin and sodium sulfadiazine. The end points of sensitivity were found later by the tube dilution method.

In a total of 158 infant patients and 82 adult personnel during a four month period E. coli 0127 was isolated from 44 infant patients and from one attending nurse. In the acute phase this type was found in practically pure culture. Shigella was cultured from 2 patients; Salmonella from 5 patients and one student nurse.

OBSERVATIONS ON THE CONVALESCENT PHASE OF ERYTHROBLASTOSIS FETALIS

Carol B. Hyman, et al. (Children's Hospital, Los Angeles) Pediatrics 16, 15-23 (1955).

Twenty infants were observed during convalescence from erythroblastosis fetalis. It was pointed out that many blood transfusions may be avoided if the rise of total body hemoglobin is noted (calculated from the blood hemoglobin concentration on the basis of 40 ml. of blood per pound of body weight). The bone marrow did not show a characteristic pattern of erythropoietic activity. There is evidence that an initial aregenerative period exists in erythropoiesis during early convalescence in that reticulocytosis is not shown and the cells are slow in becoming Rh positive.

HEMATOLOGIC OBSERVATIONS OF THE COURSE OF ERYTHROBLASTOSIS FETALIS

Jane F. Desforges, and Liam G. O'Connell (Boston City Hospital) Blood X, 802-11 (1955).

Thirty-eight patients with erythroblastosis fetalis were studied for this report. Thirty-three were due to anti-D and four to anti-A. One case had both antibodies. Eight received replacement transfusions during the first six hours after birth, five more within the first day, and eight during a period of 24 to 72 hours.

Immediate effects included a decrease in the level of bilirubin, reticulocytes and circulatory nucleated red cells in addition to a change in the hemoglobin value and platelet count. Serum bilirubin before transfusion from 1.6 to 24.6 mgs. per cent. After transfusion the level was never sufficiently high to warrant a second replacement transfusion.

Abnormalities in the osmotic and mechanical fragility of the red cells were found; more susceptibility to osmotic stress while hemolysis units were in the normal range.

EXPERIMENTAL AND STATISTICAL STUDIES ON RH ANTIBODIES

Vera I. Krieger, and E. J. Williams (Royal Women's Hospital, Melbourne) J. Lab. Clin. Med. 46, 199-224 (1955).

A series of studies was undertaken to determine the reason for some of the discrepancies which have arisen as the field of erythroblastosis fetalis has become so extensive. The studies were divided into four parts. The first three parts give a new interpretation suggested in part four of the results obtained by various techniques for the estimation of Rh antibodies:

(1) The albumin titer:
   a. In albumin titrations the effect of incomplete antibodies and saline agglutinins is additive.
   b. The concentration of Type 2 incomplete antibodies is the difference between the saline and albumin titers when the incomplete antibodies do not include blocking antibodies.
   c. The saline titer estimates excess saline agglutinins only when blocking antibodies acting by neutralization are present.
(2) The Wiener blocking test probably detects Type 1 incomplete antibodies, that is, blocking antibodies for saline agglutinins only.
(3) From the evidence presented the fifth type of Rh antibody is the only one of the five known forms which is detected by the indirect Coombs test.

AN UNUSUAL ANTIBODY PATTERN IN A CASE OF IDIOPATHIC ACQUIRED HEMOLYTIC ANEMIA

Rose Payne, et al. (Stanford University School of Medicine) J. Lab. Clin. Med. 46, 245-54 (1955).

Two unusual features of a case of idiopathic acquired hemolytic anemia are reported: (1) hemoglobinuria; (2) the serum contained an agglutinin which was active against trypsinized erythrocytes although the patient's red blood cells were Coombs' negative.

Many steps were taken to identify the patient's agglutinin. The agglutinin did resemble that found in the serum of normal persons tested against trypsinized cells but differed in that the titer was considerably higher and the agglutination was not reversible and papain-tested cells were susceptible to agglutination. That the trypsin test is a necessary adjunct in the investigation of acquired hemolytic anemia was concluded.

AUTO-ERYTHROCYTE SENSITIZATION
A Form of Purpura Producing Painful Bruising Following Autosensitization to Red Blood Cells in Certain Women

Frank H. Gardner, and Louis K. Diamond (Harvard Medical School) Blood, X, 675-90 (1955).

Four women with purpura were studied because of their unusual response to bruising. Autosensitization by the patients to their own blood was suggested. Skin testing indicated an abnormal tissue response of sensitivity to red blood cells. This response was to the factor in the red cell stroma and not associated with the hemoglobin.

INFECTIOUS MONONUCLEOSIS AND ACUTE HEMOLYTIC ANEMIA; REPORT OF TWO CASES AND REVIEW OF THE LITERATURE

Richard H. Thurm, and Frank Bassen (Mt. Sinai Hospital, New York) Blood, X, 841-51 (1955).

The complication of hemolytic anemia with infectious mononucleosis was found in two cases. In the first the anemia was sudden and the reticulocytes, erythroid hyperplasia of the bone marrow and the transient methemalbuminemia gave the appearance of a hemolytic process. The second case had an underlying Mediterranean anemia. Atypical lymphocytes and strongly positive heterophile agglutination tests confirmed the diagnosis of infectious mononucleosis. There are thirteen reported cases of similar complications.

THE LABORATORY DIAGNOSIS OF AMEBIASIS

Dr. Clyde Swartzwelder, Department of Microbiology, Louisiana State University, School of Medicine, New Orleans, Louisiana

The prevalence of symptomatic and asymptomatic carriers of amebiasis in the general population and the poor prognosis of undiagnosed hepatic amebiasis necessitate the accurate diagnosis of this infection. The objects of this presentation are to review the fundamentals upon which a correct laboratory diagnosis of amebiasis are based and to point out the common errors which may be made. Without a working knowledge of the fundamentals and a reasonable period of supervised experience, *missed* diagnoses and *misdiagnoses* of amebiasis may result. The diagnosis of amebiasis may be missed due to faulty laboratory technic. There have been some improvements in the methods for the demonstrations of *Endamoeba histolytica*, particularly in stool concentration and culture technics. Conversely, the infection may be overdiagnosed due to confusion of *Endamoeba histolytica* with normal or abnormal constituents of stools. Erroneous laboratory diagnosis may result in unnecessary medication. It also may delay the physician in arriving at a correct diagnosis of the patient's illness. There must be strict adherence to the accepted criteria for the identification of *E. histolytica* in order to avoid the pitfalls that lead to false diagnoses. Since specific diagnosis of amebiasis essentially depends upon demonstration and correct identification of either cysts or trophozoites of *Endamoeba histolytica*, the technologist plays the key role in the accurate diagnosis of this disease.

LABORATORY DIAGNOSIS OF NEMATODE INFECTIONS

Paul C. Beaver, Ph. D., Professor of Parasitology, Department of Tropical Medicine and Public Health, The Tulane University, School of Medicine, New Orleans, Louisiana.

To revise and supplement "The detection and identification of some common nematode parasites of man," Part II of Symposium on Parasitology, 1952 (American Journal of Clinical Pathology 22: 481-494), the following are deserving of special consideration: (1) choice of the concentration technic (old and new) most suitable for different types of stools and infections under suspicion; (2) estimations of worm burden by egg counts; and (3) aids in the diagnosis of non-patent and exotic infections.

ACTINOMYCOSIS AND NOCARDIOSIS

Albert L. McQuown, M. D., Co-Director of Laboratories, Our Lady of the Lake Sanitarium, Baton Rouge, Louisiana.

The transitional family group of organisms between the true bacteria (*Schizomycetes*) and true fungi (*Eumycetes*) are the *Actinomycetes*. These organisms produce gram-positive branching filaments which frequently fragment to a bacillary form. The genera *Nocardia* is aerobic and many species are acid-fast. They produce the disease nocardiosis. The genera *Actinomyces* are anaerobic and produce antinomycosis. These diseases are tuberculosis-like and must be ruled out in the differential diagnosis of any chronic infection. Identification is essential for proper care and treatment

ELUTION TECHNIC FOR IDENTIFICATION OF ANTIBODY-COATED ERYTHROCYTES

Leon N. Sussman, M.D., and Hannah Pretshold, M.S., M.T. (ASCP), Laboratory of Beth Israel Hospital, New York City. The American Journal of Clinical Pathology, Vol. 24, No. 12, December, 1954.

A technic is presented for the application of the elution method described by Landsteiner and others, to the identification of antibody coating the erythrocytes of newborn infants.

The increasing frequency of sensitization to other blood factors besides the Rh_0 factor necessitates vigilance and attention when the antiglobulin test of the erythrocyte of newborn is found to be positive.

The offending antibody can frequently be identified by study of the mother's erythrocytes and serum. Proof of this identification can be had by the elution technic, utilizing the bady's coated erythrocytes and properly selected test cells.

PREPARATION OF ANTI-A_1 (absorbed B) SERUM A SIMPLIFIED AND INEXPENSIVE TECHNIC

Leon N. Sussman, M.D., F.A.C.P., Hannah Pretshold, M.S., ASMT, The Laboratory of Beth Israel Hospital, New York City.

A simplified and inexpensive technic for making anti-A_1 (Absorbed B serum) from commercial anti A (type B) serum is described. Any amount can be made to suit the needs of the individual laboratory, and the remainder of the serum stored indefinitely in the freezing unit of a refrigerator. The cost is about one fifth of the price of commercial anti A_1 serum. It is also a more reliable serum with a stable titer.

THE GAVEL

"SERVICE THRU COOPERATION"—these words were chosen to be used in connection with the First North American Conference of Medical Technologists in Quebec City next June. Service involves others, and cooperation takes more than one, so the two go hand in hand. It is with this thought in mind that this message is written. In serving others thru the many different possible channels of cooperation, you actually are just following your chosen profession. Being a medical technologist is thinking of others first, whether it be the patient, your fellow technologist, the physician, or whoever you come in contact with. With a new year at hand let us check the supplies, replenish where necessary, and make this next year nineteen hundred and fifty six all that medical technology could wish for. To all of you a HAPPY NEW YEAR.

Barbara Isbell, M.T. (ASCP)
President

FROM OUR READERS

Mrs. Jacqueline Bahrenburg, St. Luke's Hospital, Spokane, Washington, has shared with us a copy of a talk by Walter Bridge, Ph.D., D.D., Pastor of the First Baptist Church, Spokane, directed to the graduating class of medical technology students last February. In his "The Challenge of Service," Dr. Bridge reviews the development of medical technology as a profession. He brings out points on how the profession is an opportunity for young men and women to enter a field of service that offers "a real challenge and opportunity."

THE BOARD OF SCHOOLS

The American Society of Medical Technologists enjoys a very important cooperative relationship with the American Society of Clinical Pathologists through its representation on the Board of Schools of Medical Technology. Although the activities of this Board have been described previously, we are happy to repeat the answers to several questions received from the A.S.M.T. membership.

What Is the Board of Schools?

The Board of Schools is an elected board composed of six members of the A.S.C.P. and three representatives from A.S.M.T. It is an *advisory board* to the Council on Medical Education and Hospitals of the American Medical Association.

What Are the Functions of the Board of Schools of Medical Technology?

The primary function of the Board is to serve in an advisory capacity to the Council on Medical Education and Hospitals, A.M.A. The Board encourages and assists in the establishment of new schools. It works toward the maintenance of high standards of education.

How Is the Board Financed?

The Board is financed by a budget from the A.S.C.P. This allotment covers the expenses of the A.S.C.P. members to the annual meeting; the cost of a part time secretary; postage and

printing. The A.S.M.T. pays the expenses of its three representatives attending the annual meeting, and postage.

What Is the Role of the Board in Approving New Schools?

When an institution wishes to establish a school of medical technology, the application is made to the Council on Medical Education, A.M.A. The Council then refers the application to each member of the board, via mail, for their evaluation and opinions. The application is then returned to the Council, bearing recommendations by the Board of Schools. These are based upon the opinions expressed by the nine members. It is the prerogative of the Council (*not* the Board of Schools) to grant, withhold or withdraw approval. *The Board may only recommend.*

What Is the Role of the Board in the Inspection of Schools?

When the Council could no longer assume the responsibility of examining the various auxiliary departments such as X-ray, etc., it became clear that the Pathologists would have to do their own examining. Thus, three years ago, the Board was given the responsibility of inspecting the schools of Medical Technology. Since there was no organized program to serve as a pattern, and no funds were made available, the Board had to organize its own inspection program and to solicit pathologist examiners on a voluntary basis. The Board devised a point rating system and supplied all existing schools with copies of the same. Upon the basis of the point rating system and the inspectors' reports, recommendations were made to the Council regarding each school. To date, all but 16 old schools have been inspected. The Board plans to continue this personal inspection program, 1. for each new school within a year of accepting students; 2. all schools having change of Directors; 3. schools having any change or problems which might affect the teaching program, and 4. on a 3-5 year basis for old schools.

It was upon the basis of a recommendation from the Board of Schools that the Council revised the annual report so that it permits a limited, but valuable, yearly re-evaluation of each school. This report is used also as a guide in studying various trends being observed by the Board.

What Has the Board of Schools Done for New Schools?

One of the greatest achievements of the Board was the preparation, printing and distribution of a booklet, "How to Organize an Approved School of Medical Technology" and "Recommended Records in an Approved School of Medical Technology." This booklet has brought about a noteworthy improvement in the quality of applications being received.

In addition, the Board has written 294 letters to Colleges and Universities urging the establishment of curricula leading to a

degree in Medical Technology. The response to this project has been very encouraging, both in numbers and in the reactions of the Deans.

The Board has also an exhibit entitled "Career—Medical Technology." This has been exhibited at the A.S.M.T. Convention and various other career programs. It is available for anyone interested in utilizing it.

Space does not permit more at this time, but we refer you to the February News Release for a detailed account of the 1955 meeting of the Board of Schools of Medical Technology, A.S.C.P.

ANNOUNCEMENTS

The University of Kansas School of Medicine announces its Seventh Annual Postgraduate Course in MEDICAL TECHNOLOGY, to be offered January 9, 10 and 11, 1956, at the K. U. Medical Center, Kansas City, Kansas.

The course will present recent developments and evaluations in tests for various contagious diseases; laboratory and clinical aspects of serologic tests with *T. pallidum* antigens; and subjects in hematology, including coagulation, prothrombin tests, bone marrow, auto-immunization phenomena and transfusions. A symposium at the close of each day's program will provide those in attendance an opportunity to have special problems discussed.

The distinguished guest faculty includes:

CHARLES A. HUNTER, Ph.D., Director, Division of Public Health Laboratories, Kansas State Board of Health, Topeka, Kansas.

ALBERT P. McKEE, M.D., Professor of Bacteriology, State University of Iowa College of Medicine; Member, Influenza Commission, Armed Forces Epidemiology Board; Coordinator, Sectional Research in Microbiology, U. S. Public Health Service, Iowa City, Iowa.

HAROLD J. MAGNUSON, M.D., Research Professor of Experimental Medicine, University of North Carolina School of Medicine; Medical Officer in Charge, V. D. Experimental Laboratory, U. S. Public Health Service, Chapel Hill, N. C.

The course is open to all serving in medical laboratories. A fee of $12.00 is charged. For program announcement and registration card, write: Department of Postgraduate Medical Education, University of Kansas Medical Center, Kansas City 12, Kansas.

COURSE IN EXFOLIATIVE CYTOLOGY

A course in Exfoliative Cytology will be given at the Papanicolaou Cytology Laboratory, Cornell University Medical College, March 5 to June 1, 1956. The program includes lectures, demonstrations, discussions, practice in the preparation and staining of smears, and practical instruction in the interpretation of exfoliative cytologic material.

The course is open to both physicians and technologists. Requirements for the latter include: (1) Employment or contract for future employment by an approved hospital or laboratory, with supervision of the cytologic work by an adequately trained cytologist or cyto-pathologist; (2) A course in histology, including microscopic study of tissues as well as technic. Other related courses such as hematology, gross anatomy and pathology, though not required, constitute a valuable background.

Tuition for the course is $300.00.

Inquiries from technologists should be addressed to Miss Charlotte M. Street, and from physicians to Dr. John F. Seybolt, Papanicolaou Cytology Laboratory, Cornell University Medical College, 1300 York Avenue, New York 21, N. Y.